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Mercury methylation in sediments from coastal and Sierra watersheds : implications for methylmercury mitigation in the San Francisco Bay-Delta complex

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MERCURY METHYLATION IN SEDIMENTS FROM COASTAL AND SIERRA WATERSHEDS: IMPLICATIONS FOR METHYLMERCURY MITIGATION IN THE SAN FRANCISCO BAY-DELTA COMPLEX

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ABSTRACT

MERCURY METHYLATION IN SEDIMENTS FROM COASTAL AND SIERRA WATERSHEDS: IMPLICATIONS FOR METHYLMERCURY MITIGATION IN THE SAN FRANCISCO BAY-DELTA COMPLEX

by Tom S. Kimball

The San Francisco Bay-Delta Complex is contaminated with mercury, and many fish tissue concentrations exceed US Food and Drug Administration (FDA) limits for human consumption. Much of the mercury is historic and can be traced to contaminated sediments from hydraulic mining. Today, contamination continues from two major sources: mercury mines in the coast range and gold mines in the Sierra foothills. Mercury from both watershed sources is methylated in receiving sediments within the Delta. Little is known about the relative bioavailability and chemical reactivity of this mercury once incorporated into Delta sediments. To prioritize mitigation options, this study assessed methylation efficiency (ratio of methylmercury:total mercury) at three Delta locations using laboratory and field experiments with mixed and transplanted sediment. Methylation efficiency was found to be greatest for Sierra sediment and lowest for coast range sediment. Methylation efficiency of ionic mercury was spatially and temporally variable, though during the summer was greater than for other forms of Hg (including controls). Methylmercury production was proportional to the total mercury (THg) concentration in sediments, yet efficiency of this transformation depended on receiving and source sediment. Overall, field results using *in situ* sediment transplant experiments substantiate laboratory findings. Together, these results indicate that reductions in THg are an effective strategy for the reduction of methylmercury in the San Francisco Bay-Delta Complex and should reduce biota methylmercury exposure. Due to source strength and reactivity, this study suggests that elemental mercury from abandoned gold mines in the Sierras should be the highest priority for mitigation.

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Introduction

The San Francisco Bay-Delta Complex (Delta) is immensely important for a variety of reasons: it supports 100's of fish and bird species, provides water for California from a 61,000 mi^2 watershed for a variety of uses, supports 500 million dollar per year agriculture, and has 1,000's of miles of waterways for recreational use. Thus, protecting this environment from the risks of mercury to human and wildlife health is imperative for long-term sustainability of both this system and the state of California.

Mercury contamination is a serious problem in the Delta ecosystem (Domagalski 1998, Roth et al. 2000, Heim 2003) and is threatening wildlife and human health (Davis et al. 2000, Schwarzbach et al. 2005); many fish exceed US Environmental Protection Agency (EPA) and US Food and Drug Administration (FDA) limits for human consumption (California 303(d) list 2003, Davis and Greenfield 2002). Much of the original mercury contamination dates to the mid 1800's, yet contamination continues from two major sources: mercury mines in the coast range and gold mines in the Sierra foothills (Foe and Croyle 1998, Alpers and Hunerlach 2000, Foe 2002; Figure 1). Cinnabar (HgS) mining in California between 1850 and 1981 produced about 220 million pounds of elemental mercury (Churchill 2000). In the Sierras, an estimated 3-8 million pounds of this mercury was lost to the environment during gold mining through the Hg-Au amalgam process (Alpers and Hunerlach 2000).

Once incorporated into Delta wetland sediments, inorganic Hg may be transformed to toxic monomethylmercury (MeHg) and biomagnified in the food chain. Sulfate reducing bacteria mediate methylation (Compeau and Bartha 1985, Winfrey and

Rudd 1990, Gilmore et al. 1992, Gilmore and Riedel 1995), a process occurring primarily in sediments and wetlands at low oxygen concentrations (Krabbenhoft et al. 1995, Branfireun et al. 1996, Benoit et al. 1998, Gilmour et al. 1998, Pak and Bartha 1998). Methylation increases with greater inorganic Hg loading, sulfate reduction (SR), and dissolved organic carbon (DOC; Compeau and Bartha 1984, Ramlal et al. 1985, Benoit et al. 2003). Methylmercury may also be converted back to inorganic mercury by bacteria in the sediment through bio-demethylation, or in surface waters by photo-demethylation (Marvin-DiPasquale et al. 2000, Sellers et al. 2001) and is thus constantly being created and destroyed.

Production of MeHg is known to be greater in wetland sediments than other aquatic habitats (St. Louis et al. 1994, Hurley et al. 1995, St. Louis et al. 1996). Heim et al. (2003) found that wetland habitats in the Delta methylated mercury more efficiently than other habitats surveyed. Studies of Delta wetlands found strong geographic and habitat-specific relationships between MeHg and habitat type (Gill et al. 2002, Slotten et al. 2002, Heim 2003). This suggests that factors important to the post-depositional methylation of Hg include characteristics of the depositional habitat. However, other possibilities for these observed differences in methylation include Hg speciation. This study is meant to differentiate the relative influence of habitat and speciation on the methylation of mercury.

Mercury entering the Delta (source sediment) from coast range watersheds is primarily in mineral form (cinnabar and meta-cinnabar, HgS) originating from mercury mine waste (Churchill 2000). Mercury entering the Delta from Sierra watersheds is from

gold mine wastes and consists primarily of elemental Hg (Hg^0) and species derived from Hg^0 (Hg-humics, HgCl, $HgCl_2$, and Hg^{2+}; Bloom 2002). Elemental and complexed forms of Hg, like those originating from the Sierras, are more soluble in sediment, and are more readily methylated than cinnabar forms (Bloom 2002, Suchanek et al. 2002). Based on comparisons with laboratory grade mercury as a proxy for Sierra sediment, these previous studies have also proposed that mercury from the Sierras is more biologically available for methylation than mercury from the coast range. Yet, this hypothesis was not directly tested. Little is known about the relative bioavialability and chemical reactivity of Hg from Sierra and coastal sources once incorporated into Delta sediments.

Inputs of mercury to the Delta from atmospheric deposition are thought to be relatively low (Sacramento-San Joaquin Delta Estuary TMDL for total and methylmercury 2005). Elemental mercury can volatilize from a variety of sources both local and worldwide and be emitted to the atmosphere where it may be transported on wind currents for a year or more. Elemental Hg in the atmosphere may be converted to Hg^{2+} through oxidation. Ionic Hg has an atmospheric residence time of less than two weeks due to its solubility in water, low volatility and particle reactive properties. Ionic Hg can be rapidly taken up in rain water, snow, or adsorbed onto small particles, and be subsequently deposited in the environment through "wet" or "dry" deposition. Ionic mercury deposited to the Delta may be in a form readily available for methylation, and thus may be important with respect to methylmercury production.

The methylmercury:inorganic mercury ratio in sediment is a proxy of methylation efficiency, indicating how readily inorganic mercury is converted to MeHg in sediments

(Krabbenhoft et al. 1999). Methylation efficiency is an important aspect of watershed management. It has been recommended that mercury mitigation should focus on habitats with greater methylation efficiency (e.g. some wetlands) because they are more sensitive to inorganic mercury contamination and more likely to exacerbate MeHg exposure. These aquatic habitats may be identified as mercury sensitive habitats - aquatic ecosystems in which total mercury inventories cause relatively more MeHg bioaccumulation in upper trophic level wildlife (Wiener et al. 2003).

The efficiency with which different forms of inorganic Hg are converted to the toxic MeHg in pore waters is important. Source control of inorganic mercury is a viable strategy for methylmercury mitigation. Clean up of mercury may prioritize those sources that are efficiently methylated once incorporated into Delta sediments.

Methods for assessing MeHg production (and methylation efficiency) in sediments vary and include time series measurements of anoxic slurries (Slotten et al. 2002, Suchanek et al. 2002), controlled microcosms (Compeau and Bartha 1984, Bloom 2002), radiolabel assays (Gilmore and Riedel 1995, Marvin-DiPasquale and Agee 2003), and incubated core experiments (Ramlal et al. 1985, Gilmore and Riedel 1995, Best et al. 2005). Gilmore et al. (1992) suggested that whole-core dosing experiments better represent the environment than anoxic slurries because they preserve redox gradients critical to biogeochemical processes and maintain *in situ* sulfate reducing bacterial communities. Recent Hg cycling studies (Hintelmann et al. 2002, Krabbenhoft et al. 2006) have used *in situ* Hg dosing methodology that closely mimics processes occurring in the environment in that experiments were performed at scales representative of the

entire ecosystem. Thus, both laboratory and *in situ* experiments are useful to inform the process of Hg biogeochemical cycling.

Experimental Design

Mitigation efforts for methylmercury will need to take into account total mercury concentration, source of mercury, and characteristics of the methylating habitat. Previous work suggests that mercury from the Sierras may be more easily methylated than mercury from the coast range, and that methylation of THg (measure of mercury in all forms, primarily inorganic mercury) is site specific. Whole-core dosing (laboratory) and *in situ* sediment transplant (field) experiments in this study were performed to inform and prioritize such mercury mitigation efforts.

This study examined differences in methylation of ionic mercury (Hg^{2+}) and mercury contained in sediment from geographically distinct sources (coast range and Sierra) varying widely in concentration and speciation. Net methylmercury production and methylation efficiency (MeHg:THg) was assessed at three Delta locations using transplanted sediments. Transplant material consisted of receiving sediment mixed with source sediment and Hg^{2+}.

Methylation efficiency experiments were conducted on two representative scales: 1) intact cores in the laboratory, and 2) benthic substrates in the field. Laboratory experiments consisted of sediment transplants to intact incubated whole-sediment cores. Field experiments consisted of sediment transplants to *in situ* sediment. Although both net MeHg production and methylation efficiency will likely reflect the source and thus bioavailability of THg added, determination of methylation efficiency will allow for

direct comparison between treatments because it corrects for the increase in MeHg due to increase in THg alone.

Materials and Methods

Source and Receiving Sediments

Surficial sediments from three Hg contaminated locations (source sediment) was collected in September 2003 and June 2004; one location in the coast range and two in the Sierras. These sediments were known to be contaminated with mercury from past mining activities and are representative of sources of Hg to downstream methylating habitats in the Delta: 1) Bear Creek (coast range), 2) American River (Sierras), and 3) Starr Tunnel (Sierras; Table 1, Figure 1). Sediments were stored at 1-6 °C until use (within a month).

Sediment from Bear Creek was collected near the Highway 20 bridge (39.01154° N, 122.36117° W) in the Cache Creek watershed by simply using a shovel to collect sediment from a point bar into a 5-gallon plastic bucket. Sediment from this location represents sediment contaminated from historic Hg mining activity (cinnabar) and geothermal sources of Hg (Bloom 2002).

Sediment from the American River was collected near Camp Lotus (38.68703° N, 120.91762° W). With flows in the river drawn down by hydroelectric diversion, sediment was collected with the aid of mask and snorkel: bulk sediment known to be contaminated with Hg was removed from the river bed with a shovel and processed with

2 mm sieve on shore. Sediment from this location contained visible droplets of elemental mercury and represents sediment contaminated from historic gold mining activity.

Sediment from Starr Tunnel (39.22470° N, 120.91094° W, near Greenhorn Creek in the Bear River watershed) was collected from the bottom of small pools found 10-20 meters inside the mouth of the tunnel. This sediment was collected with a plastic dustpan by skimming the top 1 cm of the sediment. Although no visible droplets of Hg were observed, this sediment represents contamination from historic gold mining activity (elemental Hg). Droplets of elemental Hg were found during collection in sediment from near the mouth of the tunnel (within the small rivulet of water exiting the tunnel). The sediment collected for use in experiments likely contained Hg that was bound to clay sediment (derived from local shale).

Sediment from the Delta (receiving sediment) was collected from Franks Tract, Cache Slough and 14 Mile Slough (Table 2, Figure 2). These sites were chosen to represent depositional wetland habitat found widespread in the Delta, allowing for greater extrapolation of results. These sites were chosen from a variety of locations visited by a previous survey of mercury and methylmercury in Delta sediments (CALFED Mercury Study; Heim et al. 2003). The selections were based on several criteria: Delta sub-region, source water, Hg load sources, THg concentrations, MeHg concentrations, methylation efficiency, and Loss on Ignition (LOI, a proxy for organic matter, Table 2). In addition, Cache Slough, 14 Mile Slough and Franks Tract represented environments inundated with Sacramento River water (chloride dominated), San Joaquin River water (sulfate dominated) and Central Delta waters (a blend of the two, respectively). Using

these sites also allowed for testing of sediment with similar total mercury concentrations (near 150 ng/g dry weight; reported as dry weight throughout report) yet varying methylmercury concentrations (0.4 to 3 ng/g), and thus methylation efficiencies (MeHg:THg ratios of 0.003 to 0.02). Previous work at these locations found that sediment consisted primarily of silt and clay sized particles.

Cache Slough receives Hg from the coast range (HgS; Table 2), though during high winter flows may receive additional Hg from the Sacramento River via the Sutter Bypass (Sacramento-San Joaquin Delta Estuary TMDL for total and methylmercury 2005). Franks Tract receives Hg from both the coast range (HgS) and Sierras (derived from Hg^0), and eroded sediment from Suisun Bay (Foe 2002) that is Hg contaminated. 14 Mile Slough receives Hg from the Sierras (derived from Hg^0).

Experimental Procedures

Generalized methods for each experimental method (both laboratory and field-based) are outlined in Tables 3 and 4. A more detailed description of each is provided below. Lab tests using incubated cores from receiving sediment location (Delta) were designed to integrate biogeochemical processes including those associated with native microbial communities. Manipulative experiments consisting of *in situ* additions to native benthic sediment with 1 m^2 area (plot) at each receiving sediment location (Delta) were designed to integrate all natural processes occurring over larger spatial and temporal scales.

Methylation efficiency was determined in receiving sediment dosed with varying forms of mercury: laboratory grade Hg^{2+} (SPEX Certified Prep stock solution) or source

sediment from coastal or Sierra watersheds with elevated Hg concentrations. Varying proportions of source and receiving sediment (surficial) were mixed (slurry) such that environmentally relevant concentrations of THg (< 1000 ng/g generally) were achieved. Mimicking a fluvial deposition event, whole-core and *in situ* sediment surfaces were topped with 1 cm of slurry that consisted of receiving sediment amended with the appropriate treatment of Hg (Hg^{2+}, Bear Creek, American River, Starr Tunnel). Slurries were prepared either immediately prior to transplant (fall 2003), or prepared a few days prior to test initiation (summer 2004 - laboratory and field experiments, stored in 10 L containers at ~1 °C as pretreatment).

Intact sediment cores (polycarbonate cylinders, 5 cm diameter, 20 cm height) used in the laboratory-based whole-core methylation experiments were collected at the receiving sediment sites in triplicate push cores to contain 10 cm of sediment and 10 cm of overlying ambient water (0.5 L). This ensured the integrity of the sediment/water interface and infaunal and microbial communities in the top few centimeters of sediment, thus maintaining critical biogeochemical gradients. Surficial sediment at the receiving sediment sites (top 1 cm) was collected either by SCUBA or by using the Sludge-O-Matic (a device developed by Moss Landing Marine Laboratories to remotely sample the surficial 0.5 cm of sediments; Heim 2003). Collections of cores and surficial sediments were made using SCUBA in Fall 2003 and Summer 2004. Surficial sediments were stored at 1-6 °C until use. Cores were held at temperature characteristic of the Delta (20 °C) throughout the experimental procedure. Water quality parameters temperature, EC, pH, and DO were measured at the time of collections.

Incubated cores were equilibrated to laboratory conditions for 7-18 days prior to the addition of slurry. In the lab, the overlying waters in the cores were aerated at 20°C under 16 hr light (5-8 μE/m^2), 8 hr dark conditions. To determine peak Hg^{2+} methylation, a preliminary time-series was performed in which individual cores were terminated on days 0, 1, 2, 4, and 8 of incubation following slurry addition. This time course experiment involved cores and surficial sediment from Franks Tract only. Sediment from the top 1-2 cm of the cores was collected at the end of the incubation period and frozen until analyses. The depth of greatest methylation was determined in the timing experiment by testing sediment from both 0-1 cm and 1-2 cm portions. This initial test indicated that greater methylation efficiency occurred in samples from the surface (0-1 cm) and thus cores from later experiment were sub sampled at 0-1 cm only. Later lab experiments conducted tests with 1) triplicate cores, 2) surficial sediment from all three Delta locations, and 3) an incubation time of 8 days. Tests conducted under cold conditions (~1°C) represent mercury-dosed sediment sampled from bulk 10 L containers with six-day incubation at wet-ice temperatures (pre-treatment of sediment slurries for summer 2004 experiments).

In situ sediment transplant experiments initiated in July 2004 were designed to allow manipulation of 1 m^2 of natural sediment (Figure 3). Cylindrical polyethylene sheeting was attached to a polyethylene coated metal hoop (1 m^2) and used to isolate the surface of the benthos and top 1 meter of overlying water. One such bag/hoop assemblage was used for each treatment: Hg^{2+}, Bear Creek, Starr Tunnel, and control, at each of the three sites. Cleaned marker sand (white quartz, 0.3 cm deep layer) and

sediment slurries (1 cm deep layer) were then added to the top of the benthos (plot) through a port in the sheeting. The layer of marker sand was added to help with later identification of the sediment amended with Hg and control for post addition erosion or deposition. The sheeting enclosure was removed approximately 24 hours after deployment (after settling of sediment), thus leaving treated plots completely exposed to the environment - only the ring remained for demarcation purposes. These *in situ* plots were subsequently sampled with 5 cm or 10 cm diameter polycarbonate cores at 1, 2, 4, and 11 weeks. Cores were subsampled in the field and stored on dry ice during transport to the laboratory.

Sediment samples for mercury analyses were handled with clean technique (Puckett and van Buuren 2000) and stored frozen. Total mercury in sediments was analyzed by cold vapor atomic absorption spectroscopy (CVAAS) using a Perkin Elmer Flow Injection Mercury System (FIMS-100) following digestion with aqua regia and reduction with stannous chloride (Bloom 1989). MeHg in sediments was extracted by acidic potassium bromide into methylene chloride to separate MeHg from the sediment-water matrix. An ethylating agent was added to each sample to form a volatile methyl-ethylmercury derivative, and then purged onto carbon traps as a means of preconcentration and interference removal. The sample was then isothermally chromatographed, pyrolitically broken down to elemental mercury, and detected as using a cold vapor atomic fluorescence spectrophotometer (Tekran CVAFS Mercury Detector 2500; Bloom 1989, Bloom 1997).

Sediment loss on ignition (a proxy for organic matter content) and particle size was measured in representative sediment samples from each receiving sediment location for each season sampled. These ancillary parameters were collected to test their relative importance with respect to methylation efficiency. In addition, assessment of sediment size can help verify that the Delta locations sampled were of similar geochemical composition. Sediment particle size analyses were conducted using a Beckman-Coulter LS 1230 laser particle size analyzer. LOI was conducted using 20 mL glass vials and sediment was combusted at 550ºC until weight was constant.

SYSTAT 10 was used for statistical analyses. Regression analyses were performed with the dependant variable MeHg and the independent variable THg from the Hg^{2+} treatments. Mean MeHg:THg ratios were compared using ANOVA and Fisher's Least-Significant-Difference Test (alpha = 0.05).

Results

Environmental parameters of water at study sites during the time of sediment collection (Fall 03 and Summer 04) and during the Summer 04 field experiment incubation period were characteristic of freshwater dominated Delta wetlands (Table 5). Sediment temperature (surface) ranged from 13.1-27.0 °C (Table 5). Benthic habitat was representative of shallow (3 m), open water wetland found widespread in the Delta and contained predominately fine-grained sediment and 6-13 % organic carbon (as measured by LOI; Table 6). LOI was highest for 14 Mile Slough, 10.8 ± 3.9%, and lower for Franks Tract and Cache Slough, 7.9 ± 1.1% and 5.9 ± 0.2%, respectively.

Cache Slough and Franks Tract sediment was clayey-silt; ~20% clay and ~65% silt (by volume; Table 6, Figure 4). Sediment from 14 Mile Slough was more diverse size (silty-sand); by volume ~5% clay, ~40% silt, and ~55% sand-sized, and contained more organic matter. Particle size analyses of the sediments from the three sites (Figure 2) after treatment with hydrogen peroxide indicated that for 14 Mile Slough, the majority of the organic matter was larger sized (sand-sized), whereas for Cache Slough and Franks Tract, the majority of the organic matter was smaller sized (silt-sized; Table 6, Figure 4).

High concentrations of mercury were found in source sediments: Bear Creek, 2,500 ng/g; Starr Tunnel, 33,000 ng/g; and the American River, 25,000,000 ng/g (Table 1). Sediment from Bear Creek and the American River were typical riverine silty-sand sediment. The droplets of Hg^0 (1-2 mm in diameter) in the American River sediment were seen intermixed with the sediment. Though droplets of Hg^0 were observed in sediment panned from the tunnel mouth (mixed gravels and sands), none were observed in sediment collected from inside the abandoned sluice shaft (used in tests). Mercury in this contaminated sediment was presumably complexed with clay.

Whole-Sediment Incubated Core Experiments

Net production of MeHg occurred rapidly - within one day - in treatments amended with Hg^{2+} in a preliminary experiment conducted to determine the sequence of methylation (Table 7, Figure 5). Treatments in this test were amended with ~320ng/g Hg^{2+} (460 ± 52.8 ng/g dosed, 137 ± 9.4 ng/g control). Net MeHg production increased 12 times from 1.75 ng/g to 20.40 ng/g within one day and remained elevated for the duration of the test. Peak methylation efficiency (0.064) occurred on day 8. Comparison

of 0-1 cm portion with 1-2 cm portion indicated that the bulk of MeHg was found in the surficial sediment: 24.74 ± 4.74 ng/g in the 0-1 cm layer versus 4.97 ± 1.54 ng/g in the 1-2 cm layer (Table 7). In this test, methylation efficiency remained low in both the control (0.013 ± 0.0015) and Bear Creek (0.0106 ± 0.004) treatments.

Results from whole-core incubation methylation experiments conducted in the laboratory during summer (2004) and fall (2003) indicated that ambient THg concentrations in sediment from each receiving sediment location, and thus controls, were similar across both spatial and temporal scales (Table 8, 9). Net production of MeHg and thus methylation efficiency varied by both site and season (Table 8, 9, Figure 6). In the summer, methylation efficiency was highest for 14 Mile Slough (MeHg:THg = 0.0091: p=0.001, 0.002) and lower for Cache Slough and Franks Tract (MeHg:THg = 0.0041 and 0.0035, respectively: p=0.602; Table 9). In the fall, controls exhibited significantly different methylation efficiency in all locations ($p<0.001$; Table 8). Methylation efficiency was greatest in Franks Tract (0.0084) and least in Cache Slough (0.0026).

Between seasons, the greatest difference in ambient methylation efficiency was observed in Franks Tract: greater in the fall than it was in the summer (2.4 times increase, $p<0.001$; Table 8,9). Although with less relative change, the opposite trend was observed at the other two locations: methylation efficiency was greater in the summer than fall in both Cache Slough and 14 Mile Slough (1.6 times increase, p=0.013, and 1.4 times increase, p=0.136, respectively).

Spatial and temporal trends in MeHg production and methylation efficiency in control treatments mimicked those in treatments dosed with Hg^{2+} (Table 8, 9, Figure 6, 7). For example, methylation efficiency in Hg^{2+} treatments was markedly greater in the summer for Cache Slough and 14 Mile Slough (11.2 times increase, $p<0.001$ and 11.9 times increase, $p=0.002$, respectively) and methylation efficiency was greater in the fall than summer for Franks Tract (1.4 times increase, $p=0.056$). Within season the order of methylation efficiency in controls was the same as that in treatments dosed with Hg^{2+}.

Methylation efficiency in Hg^{2+} treatments during the summer were significantly increased with respect to controls: 3.2 times increase Franks Tract, 6.5 times increase Cache Slough, and 7.5 times increase 14 Mile Slough ($p<0.001$ all; Table 9, Figure 7). Conversely, methylation efficiency during the fall was linear over the range of Hg^{2+} concentrations tested (Table 8, Figure 6, 7). The regression lines indicating methylation efficiency during the fall for all three Delta locations (Figure 6) were statistically different ($p<0.001$). The slope (MeHg:THg, methylation efficiency) was highly variable between locations and indicated about an order of magnitude difference; lowest for Cache Slough (0.002), and highest for Franks Tract (0.018, Figure 6).

In the cases of Cache Slough and 14 Mile Slough in the fall, methylation efficiency was not significantly different between Hg^{2+} and control treatments: $p=0.165$, 0.614, 0.512 and $p=0.265$, 0.619, 0.225, respectively (Table 8). Although 89% (r-squared = 0.89, Figure 6) of the variability in MeHg was explained by THg in Franks Tract sediment (fall), methylation efficiencies in Hg^{2+} treatments were significantly greater than in the control (Table 8, Figure 7, $p=0.024$, 0.007, 0.006) at this site.

Methylation experiments conducted using whole-sediment incubated cores with Hg amended treatments indicated that methylation efficiency followed the order of Hg^{2+} > American River (Sierra) > Starr Tunnel (Sierra) > Bear Creek (coast; Table 8, 9, Figure 8a,b). In all cases net methylmercury production increased with the addition of Hg of any form (Table 7, 8, 9).

Concentrations of inorganic mercury in treatments with added Hg from Bear Creek and Starr Tunnel were near the intended 5-fold increase in concentration, though concentrations in sediment dosed with Bear Creek was more variable (lower than expected in fall tests, and slightly elevated in summer tests; Table 8, 9). Methylation efficiencies in Bear Creek treatments during the fall were not significantly different than respective controls ($p=0.340,0.100,0.710$, Table 8). Conversely, in the summer, methylation efficiencies in Bear Creek treatments were statistically less than controls ($p<0.001$ all three), a decrease of 7.0, 1.8, and 5.3 times, for Franks Tract, Cache Slough and 14 Mile Slough, respectively (Table 9). Methylation efficiencies in Starr Tunnel treatments were also statistically less than the controls during the summer ($p<0.001$ all); a decrease of 2.3, 1.4, and 1.4 times, for Franks Tract, Cache Slough and 14 Mile Slough, respectively (Table 9).

For all three Delta locations, methylation efficiencies in Starr Tunnel treatments were not significantly different than in Bear Creek treatments (summer, $p=0.057, 0.759, 0.462$). Conversely, MeHg:THg ratios in Hg^{2+} treatments were over an order of magnitude greater than those in Bear Creek ($p<0.001$) and Starr Tunnel ($p<0.001$) treatments (summer).

Treatments amended with American River source sediment contained higher THg concentrations (Table 8) due to higher than expected concentrations of THg in this source sediment. Significantly greater MeHg was produced in receiving sediments from all sites amended with American River source sediment ($P<0.001$ all, Figure 9a). Yet, net methylmercury production was highly variable between locations despite amendments with similar concentrations of Hg from the American River (Table 8, Figure 9a). Net MeHg production in the American River treatment for Cache Slough was very low (1.43 ng/g) compared with production for American River treatments for 14 Mile Slough (24.4 ng/g and 137 ng/g, respectively). In these treatments, net MeHg production increased 106 times in Franks Tract, 29 times in 14 Mile Slough, and yet only 5.5 times in Cache Slough.

Despite elevated THg concentrations, the methylation efficiency in the Franks Tract treatment amended with American River sediment was not statistically different than those in Hg^{2+} treatments ($p=0.509$, 0.193, 0.189; Table 8, Figure 8b) and was similar to methylation efficiency in the control (Table 8, Figure 9b). Conversely, at the locations Cache Slough and 14 Mile Slough, methylation efficiencies in treatments amended with American River sediment were significantly lower than controls ($p<0.001$ both), possibly due to the high concentrations of THg.

In a mass-by-mass comparison, far greater MeHg was produced in treatments amended with American River sediment than with Bear Creek or Starr Tunnel sediment. American River treatments produced ~170,000 ng of MeHg for every gram of American River sediment, whereas treatments amended with Bear Creek and Starr Tunnel sediment

produced only ~6.4 ng MeHg/g Bear Creek sediment and ~72 ng MeHg /g Starr Tunnel sediment (Figure 10).

Results from laboratory tests in the summer performed at lower temperature (pre-treatment with cold temperatures) were consistent with those conducted at 20° C. Net MeHg production in all treatments dosed with Hg was similar in tests under warm (20°C) and cold (1°C) conditions (Table 9, 10). Yet, the relative magnitude of MeHg production varied with temperature; in 3 of 9 cases MeHg production was greater at 1°C than at 20°C.

In Situ Sediment Transplant Experiments

The location of quartz sand within the sediment depth profile was used to help determine the depth of the added sediment layer (and thus amended Hg). Experimental mercury and sand was found together at varying depths due to post-depositional sedimentation (Table 11). Sediment deposition rate (derived from accumulation of new sediment above marker sand) over the summer (time period of the field experiment, 11 weeks) at each of the Delta locations was variable; greatest at 14 Mile Slough (0.7 mm/day) and less for Cache Slough (0.3 mm/day) and Franks Tract (0.2 mm/day). Therefore, samples analyzed for methylation efficiency were from greater depths for 14 Mile Slough (3.5 – 8.5 cm), and less depth from Cache Slough (1.5 – 3.5 cm) and Franks Tract (1.5 – 2.5 cm).

In order to identify the sediment layer with amended Hg, THg was analyzed in 0.5 cm portions through a majority of the core profile. The portion with a clear increase in THg - attributed to experimental Hg - was then analyzed for MeHg to calculate

methylation efficiency (Figure 11). At week 11, experimental mercury was not found in 2 of the 9 plots amended with THg (Franks Tract Hg^{2+} and Starr Tunnel amended treatments). Sediment Hg concentrations from earlier sampling events (one, two, and four weeks; only collected at one depth) were in general not consistent with expected THg concentrations, thus the sampling procedure was modified at the time period corresponding to 11 weeks to include sequential 0.5 cm portions. However, where elevated THg was found in these earlier samples, and where appropriate, MeHg was analyzed (Table 12). Total mercury in sediment retrieved from treatments amended with Hg ranged from 200 ng/g to 1130 ng/g (Table 11, 12) and was from a combination of sampling events (weeks one, two, four, and eleven). Controls consisted of sediment to which quartz sand was added from similar depths, but were otherwise not amended.

Results from field experiments support laboratory findings conducted with concurrently prepared transplant sediment. Net MeHg production was generally greater in treatments dosed with Hg of all forms than in controls. The order of net methylation rate remained consistent (Hg^{2+} > Starr Tunnel > Bear Creek). Also consistent with lab findings, observations from field tests indicated disproportionately greater methylation efficiency in treatments dosed with Hg^{2+} and lower methylation efficiency in Bear Creek and Starr Tunnel treatments (Figure 8, Table 12). Methylation efficiencies in Hg^{2+} amended treatments were greater than in controls (9.5 times increase at weeks 2 and 4; and 0.6 times increase at week 11), whereas they were less than controls in Starr Tunnel (8.5 times decrease) and Bear Creek (2.2 times decrease) amended treatments at week 11 (Figure 8). Mean methylation efficiencies in Starr Tunnel treatments were not

significantly different than in Bear Creek treatments (n=3, p=0.142). Unlike lab results in which Bear Creek and Starr Tunnel treatments produced greater net MeHg than controls in all cases, results from the field indicated that in about half the cases net MeHg produced in these treatments was less in controls.

Due to sedimentation, methylation efficiencies for each treatment in the *in situ* sediment transplant experiments were collected from sediment at depth rather than from surficial sediment like that in laboratory whole-core incubation experiments. Field results indicated that methylation efficiency decreased with depth (Table 12): methylation efficiencies in Hg^{2+} treatments decreased by a factor of 2.5 with depth (Cache Slough and 14 Mile Slough, 2 or 4 week compared with 11 week; Figure 12), and in controls decreased 3.3 times with depth (14 Mile Slough). For these two locations, methylation efficiencies observed in the field experiments were less than those from the laboratory experiments (14 Mile Slough, all four treatments; Cache Slough, Hg^{2+} and Starr Tunnel treatments) thus indicating that burial in the field decreased methylation efficiency. Conversely, results from Franks Tract (all four treatments) indicated that methylation efficiencies were greater in the field than the lab. In this case the sediment amended with Hg was found on the surface of the Franks Tract field experiment. With respect to decreasing methylation efficiency with depth, the greatest change was observed in the Hg^{2+} treatments in comparisons between the lab (surface) and *in situ* (at depth) experiments (Cache Slough and 14 Mile Slough, Figure 12, Table 12). In the case that Hg^{2+} was found at the surface of the *in situ* experimental plot (Franks Tract), methylation efficiency was greater in the field than the lab. Relative to controls, THg of all forms was

less available for methylation in the *in situ* experiments after 11 weeks of incubation than in whole-core dosing experiments conducted with concurrently prepared sediment (Table 9, 12).

Discussion

Consistent with previous observations in the Delta, this study indicates that MeHg production in sediment is generally greater in the summer than fall (Gill 2002, Heim 2003). Yet, seasonal and spatial trends in methylation efficiency observed in this study cannot be completely explained by the variables grain size, LOI (a proxy for organic carbon), or temperature. Thus other factors, or combinations of factors, appear to control MeHg production. Franks Tract and Cache Slough sediment, though nearly identical with respect to grain size, methylated mercury with varying efficiency, particularly in the fall. Differences in LOI may explain variability between seasons in methylation efficiency in controls; greater LOI during the summer than fall for Cache Slough and 14 Mile Slough corresponded with greater methylation efficiency during the summer. Similarly, lower LOI during the summer than fall for Franks Tract controls corresponded with lower methylation efficiency. Although differences in LOI may help explain variability in methylation efficiency observed between locations in the fall, this was not the case during the summer. In particular, methylation of Hg^{2+} during the summer could not be explained by LOI – greater LOI did not correspond to greater methylation efficiency in all cases.

Whole-sediment incubated core experiments performed during the summer and fall were conducted using similar temperature (20°C), thus seasonal differences in methylation efficiency cannot be due to temperature at the time of the tests. Incubations conducted during the summer at both cold (~1°C) and warm (20°C) temperatures indicated that MeHg production can occur at cold temperatures. This is similar to findings from a previous study (Marvin-DiPasquale and Agee 2003). In these studies, the authors observed decreased MeHg degradation with colder temperatures and thus greater methylation/demethylation ratios under cold conditions. This study found a 150% increase in net MeHg production in control sediments under cold conditions also suggestive of lower demethylation rates at cold temperatures. This indicates that temperature may have a secondary, yet important role in controlling seasonal patterns in net MeHg production.

Cold temperatures used in this study as part of the pretreatment for the laboratory incubations (conducted at warmer 20°C) could have caused the demethylation process to slow, thereby allowing for a net increase in MeHg in the control sediments. Methylation efficiency, however, increased 34% in Hg^{2+} treatments conducted at 20°C versus at ~1°C. This is in contrast with previous findings (Marvin-DiPasquale and Agee 2003) because it suggests greater net methylation at warmer temperatures for mercury that is available for methylation. Either available Hg is less readily demethylated at colder temperatures, or it is selectively methylated at warmer temperatures. This study suggests that warm temperatures following a cold treatment (such as those that arrive in the early summer in the Delta) may allow for the observed phenomena to occur *in situ*. This may explain why

methylation efficiency increases during the summer in the Delta and could suggest seasonal patterns in consortia of sulfate reducing bacteria with greater or lesser ability to methylate/demethylate Hg.

A second explanation may be simply that pore water sulfide (H_2S) concentrations (implicated in the inhibition of methylation at higher concentrations, Benoit et al. 1999) are low in the spring/summer and increase as sulfate reduction rates continue to remain high during the warm season (Marvin-DiPasquale and Agee 2003). Large sedimentation events during the winter deposit new sediment likely to be low in sulfide. As temperatures increase in the spring, so should the activity of sulfate reducing bacteria. Methylation may occur uninhibited by greater sulfide concentrations in the spring prior to sufficient sulfide build-up. Thus, the disproportionate availability of Hg^{2+} in the summer may be in part due to temperature and hydrogeomorphological related mechanisms.

Substantial methylation of mercury was observed to occur within one day in the initial laboratory experiment demonstrating that mercury methylation occur on time scales of less than a day. Mercury was methylated under similar environmental conditions as found in nature by using intact sediment cores and mimicking fluvial and estuarine sediment deposition. This indicates that within the Delta, methylation in sediments may be occurring on time scales of hours to possibly even minutes. It also suggests that both 1) the daily scour and re-deposition of sediment by tidal processes in deltas and estuaries, and 2) the ongoing and seasonally fluctuating fluvial processes of scour and deposition occurring in the upstream Delta, are mechanism that provide for a unique and dynamic habitat that is conducive to methylation. Thus, biogeochemical

gradients in sediment that support methylation are established on time scales relevant with respect to natural processes associated with large (storm-surge bed-sediment transport) and micro-scale (sub-centimeter scour and deposition events) geomorphology and bed dynamics related to both seasonal climatic fluctuations and daily tidal events. Hydrogeomorphology and biogeochemistry of tidal and fluvial influenced benthic habitats in the Delta together also provide for an environment where newly deposited material low in sulfide is rapidly deoxygenated in a reducing environment. Sulfate reduction, and thus methylation by sulfate reducing bacteria, occurs uninhibited prior to the build-up of sulfide known to inhibit methylation at higher concentrations (Benoit et al. 1999).

In a survey of methylation efficiency for a CALFED study (Heim et al. 2003) wetland habitats methylated mercury with greater efficiency (~ 0.02) than other habitats (~0.002). In wetlands, THg explained 50% of the variability in MeHg, however, the results of this study indicate a stronger relationship between MeHg and THg in wetland sediment and suggest that open water wetland habitats in the Delta methylate mercury with similar efficiency as other wetlands. The linear relationship between Hg and MeHg observed in the fall and high r-squared values (0.89 – 0.98) of these regressions indicate that net MeHg production is controlled by inorganic mercury concentration at this time of the year. Further evidence that concentration of THg controls net MeHg production is that in all cases - both in summer and fall - treatments amended with THg exhibited greater net MeHg production than controls. Combined, this data indicates that reductions

of inputs of inorganic Hg to the Delta would decrease MeHg production and thus biotic exposure to MeHg.

Resource managers do not necessarily have the means to specifically manage sediment once it enters the Delta. Unfortunately, this contaminated sediment accumulates in habitats known to methylate mercury. Current management practices do not control for the location of sediment deposition (and thus deposition of THg) within the Delta with MeHg minimization in mind. Rather, THg entering the Delta accumulates as a function of natural and anthropogenic factors such as storm events, tidal influences, vegetation, flow management and channelization. The ultimate depositional fate may occur in a variety of habitats with varying methylation efficiencies and may lie beyond the resource manager's ability to control. Thus, reduction of THg to the Delta as a whole has the opportunity to make substantial difference in decreasing MeHg production on an ecosystem wide scale by decreasing the overall amount of mercury available for methylation.

In cases where the sedimentary habitat within the Delta may be controlled or manipulated it is fundamental to understand the factors that control differences among the sites in methylation efficiency such as those observed in this study. For instance, the conditions at Cache Slough during the fall are not conducive for methylation. During the fall, controls at this site had nearly an order of magnitude less methylation efficiency than at Franks Tract. In addition, the near 10 part per million amendment to Cache Slough of elemental Hg from the American River produced an extremely low quantity of net MeHg, about two orders of magnitude less than produced in a similar treatment at Franks Tract.

Conversely, Franks Tract sediment produced a large amount of MeHg, and methylated the Hg from the American River with similar efficiency as that in controls and Hg^{2+} treatments. Combined, these results suggest we need to develop better understanding of the variables controlling these differences in order to control MeHg production in cases where we do have the ability to manipulate the environment.

Many factors act to control mercury methylation in sediments including the amount of inorganic Hg, organic carbon, sulfate reduction, temperature, and pH. In addition to these factors, the chemical speciation of mercury plays a fundamental role in net methylation rate. In this study, under most prevailing conditions, methylation efficiency was greatest for ionic laboratory-grade mercury and lowest for mercury derived from the coast range mines following the order Hg^{2+} > American River (Sierra) > Starr Tunnel (Sierra) > Bear Creek (coast range), thus providing evidence that the form of inorganic mercury controls net MeHg production. During the summer, methylation efficiency in Hg^{2+} treatments exceeded those in controls whereas methylation efficiency in treatments amended with other forms (Bear Creek, Starr Tunnel) were less than in the control. These are indications that during the summer both the amount and form of THg limit MeHg production. Yet, in the fall, linear relationships observed in treatments with all types of Hg (Hg^{2+}, Bear Creek, American River) suggests that MeHg production at this time is limited by THg concentration alone. Together, these results suggest that seasonal variability of methylation in the Delta is due to both the amount of THg available for methylation, and the form of this Hg, thus indicating seasonal variation in both the amount and source of THg to the Delta. Thus, seasonal differences in the

amount of and form of Hg entering the Delta may provide an explanation for the spring/summer peak in methylation previously observed by other researchers.

This research also suggests that spatial differences in methylmercury in the Delta ecosystem are a function of both form and concentration of THg. However, these differences may not be explained by form and concentration alone. This research is consistent with findings by other researchers (Benoit et al. 1999) that the dominant form of Hg available for methylation is neutral mercury sulfide species [e.g. $Hg(SH)_2$] in sulfidic pore waters. Recent research (Jay et al. 2000, Jay et al. 2002) indicates that polysulfide mercury species are not as readily methylated due to their charge and thus permeability through biological membranes. Clearly, the sulfur cycle plays a major role in controlling the charge, and thus availability for methylation, of mercury species. In the Delta, seasonal differences in methylation may be due to conditions associated with the sulfur cycle that favor neutral mercury species in the spring, and charged mercury species in the fall. Thus, the sulfur cycle, combined with amount and form, may help explain results and apparent variability in net methylmercury in the Delta.

Benoit et al. (1999) found that neutral mercury sulfide complexes control the availability of Hg for methylation. In the presence of greater sulfide, they predicted that available Hg is bound to sulfur species and made less available for methylation if the compound is charged (e.g. $HgSH^-$). Previous studies have found that sulfate concentrations in Delta sediment increase during the fall (Marvin-DiPasquale and Agee 2003). Thus a plausible explanation for the greater availability of Hg^{2+} during the summer demonstrated in this study may be the decreased influence of sulfide such that

the added Hg is bound with sulfur or chlorine as neutral species, rather than as charged polysulfide species. Similarly, the linear response in methylation observed in the fall in Hg^{2+} treatments may be a function of greater sulfide during this time providing an environment where available Hg was bound as charged mercury polysulfides and thus of similar (lesser) availability for methylation as that Hg in the controls. In addition, during the fall, Bear Creek (HgS) amended treatments exhibited similar methylation efficiency as the control suggesting the dominant form of Hg methylated during this time is cinnabar. Thus, differences in methylation efficiency between locations during the fall, when response to addition of Hg^{2+} was linear, cannot be explained by differing reactive mercury concentrations. Measurements of reactive mercury - as an indicator of methylation efficiency - may not prove to be the most relevant species. Rather, it appears that at times mercury of a variety of forms is methylated with consistent efficiency.

Lower methylation efficiency observed in the summer for both Bear Creek and Starr Tunnel treatments and greater methylation efficiency in Hg^{2+} treatments is consistent with the of absence of sulfide inhibition during this time. During the summer, the majority of the Hg in Bear Creek and Starr Tunnel treatments may have been bound to sulfur as HgS or sorbed to the surfaces of clay minerals, respectively, and thus not in solution. This may be why these forms of mercury were methylated with lower efficiency. Lower methylation efficiency in the cinnabar (Bear Creek) treatments suggests either the presence of 1) a charged mercury sulfide species, 2) lack of dissolution of cinnabar-bound mercury at this time, or 3) neutral species other than HgS are more readily methylated (e.g. $HgCl_2$). In addition, when sulfide is present, such that

available Hg is bound to form neutral HgS, this form is the dominant form methylated. Thus, in surficial sediments, there appears to be a constant base-rate of methylation that may represent the methylation of charged mercury sulfide species (e.g. fall). If, then, conditions allow for neutral mercury species to be formed (e.g. summer), these forms are methylated with greater efficiency. For this to occur, a source of reactive mercury must be present to form a neutral species. Where sulfide inhibition occurs, methylation appears to be more a factor of THg concentration and other unknown factors than of amount of reactive mercury. In this case, a source of reactive Hg to surficial sediment does not result in greater methylation efficiency.

In general, concentrations of Hg control MeHg production (regardless of season or sulfide), whereas form controls MeHg production in the absence of sulfide control. In the fall, methylation efficiency at Cache Slough and 14 Mile Slough is controlled only by THg concentration, whereas during the summer, methylation at all locations is controlled by form and concentration of Hg. In addition, methylation efficiency at Franks Tract was controlled by both form and concentration in the fall and summer (Hg^{2+} was methylated with greater efficiency than Hg in the control during both seasons). This suggests that 1) sources of readily available Hg (e.g. HgCl, $HgCl_2$) to the Delta during the spring/summer may have greater contribution to MeHg in the system and thus exposure of biota to MeHg, and 2) that Franks Tract is susceptible to sources of available Hg during the fall as well as the summer. This susceptibility may be a function of either a source of sulfate in the fall (sea water intrusion), or conditions that allows for minimization of sulfide despite sulfate reduction.

Ionic mercury added to sediments in this study likely immediately combined with native anions to form new mercury species. If so, then the results represent the relative availability of these new species, and indicated that they were only more available for methylation than the native mercury during the summer (and not the fall). Although these Hg species may be measured as reactive mercury (by stannous chloride reduction method, Domagalski 2001), it is clear that at times these newly formed Hg species are widely variable with respect to their availability for methylation. As such, caution should be used when interpreting the relevance of reactive mercury concentrations. It is likely that at times reactive mercury may be present, but rendered less available due to geochemical conditions. Further, interpreting the relevance of reactive mercury is even more complex due to the transient nature of these geochemical conditions. Thus, it may be difficult to ascertain the relevance of reactive mercury especially when comparing sediments with differing biogeochemical conditions and sources of variable forms of mercury. What we need to know is if the species of mercury that is formed when ionic mercury is present - and biogeochemical conditions are present that methylate this newly formed mercury species at the same rate as background – is measured as reactive or not by stannous chloride reduction.

That similar habitats within the Delta vary widely in methylation efficiency suggests controlling mechanisms that are heterogeneously distributed on a large geographic scale. Data suggests that methylation is controlled by not only concentration and form of Hg, but also additional factors other than temperature, percent organic carbon, or grain size (results this study). Cache Slough sediment appears to have severe

inhibition of methylation in the fall. This may be in part due to the lesser competing material for sulfide to bind to. Less organic carbon and more clay at Cache Slough (natural conditions at this site) may provide an environment where sulfide could preferentially bind to Hg due to less competition with organic compounds and desorption from the surface of clay minerals to bind with sulfur. In this case the form of Hg present, HgS, is less available for methylation than either other neutral species or ionic mercury. But this alone cannot explain the very low net MeHg produced when amended with Hg from the American River. It is plausible that lower sulfate reduction rates also limited MeHg production at this site during the fall (as indicated by lower LOI). Franks Tract and 14 Mile Slough may have had a source of sulfate during the fall (sea water intrusion, and the San Joaquin River, respectively). It appears that a combination of form and concentration of Hg, and other factors associated with the sulfur cycle (such as sulfate reduction, sulfide inhibition, and substrate available for combining with sulfur) are at work together. Further studies need to be conducted to elucidate the reasons for this such that when possible, these conditions may be used to reduce methylation in waterbodies that resource managers have the ability to manipulate.

In the fall, data from Franks Tract indicate that although the relationship between THg and MeHg was linear, Hg^{2+} was methylated with greater efficiency than THg in the control. In addition, this location had the greatest methylation efficiency in the control. Together, this points to a source of available Hg in the fall at this site. This could very well be sediment from Suisun Bay. Mercury contamination in this sediment was attributed to deposition from historic gold mine waste (Hornberger et al. 1999). Further,

Franks Tract controls exhibited lesser methylation efficiency in the summer than fall, suggesting that at this site the source of available Hg was greater in the fall. This is consistent with hydrogeology of the system. Decreased flows in the fall would allow greater saltwater intrusion and thus greater influence of sediment that is eroded downstream and then carried upstream by tidal processes.

Greater methylation efficiency in Hg^{2+} treatments during the summer was similar in magnitude to the greater methylation efficiencies in wetlands (Heim 2003) suggesting that the principal form of Hg methylated in wetlands is an available form similar to Hg^{2+}. During this time, greater methylation efficiency occurred in the controls compared to Bear Creek and Starr Tunnel amended treatments and thus suggests a source of available Hg in the summer in the Delta. This work is consistent with Hurley et al. (1995) who found that MeHg production increased in the spring from watersheds with more wetland area. These results indicate that available mercury may be the predominant form methylated during times when sulfide is not sufficient to bind with Hg to form HgS.

This study suggests that the enhancement of wetland methylation is due to two factors 1) lack of sulfide inhibition, and 2) a source of Hg available for methylation such as Hg^{2+}. Greater input of detritus to the benthic sediments may explain both of these phenomena. Wetlands generally have greater input of detritus to the benthic sediments due to both the trapping of exogenous sources and greater endogenous production of organic material. Mercury present in dying and decaying plant and animal matter (organic detritus) is an available form, and due to increased deposition, this detritus is rapidly placed into an early anoxic zone in the sediment where methylation can occur

(Best et al. 2005) prior to sufficient sulfide building for binding with Hg. Similarly, plant roots in wetland sediment provide microhabitats in which both anoxic and sulfide-free conditions are likely to exist, and have been shown to promote methylation (Mauro et al. 2001).

Treatments with ionic mercury show a linear response in MeHg production (Fig. 6). However, methylation efficiency was on average 63% greater in the lowest Hg^{2+} dose (2X) than the highest dose (8X) when calculated as the change in concentration from controls (control corrected methylation efficiency; ΔMeHg:ΔTHg). This differs from previous observations indicating a linear response of MeHg production with increasing THg concentrations up to 1,000 ng/g THg (Bloom 2002) and suggests that Hg^{2+} is methylated more efficiently at *in situ* (lower) concentrations. This trend was also observed in treatments amended with Bear Creek (Table 8). In these experiments, treatments amended with Bear Creek sediment contained relatively low THg concentrations (about 17 ng/g THg added). Although net MeHg production increased by 0.28 ng/g (compared to controls), the resultant MeHg:THg response was difficult to assess due to the relatively greater proportion of ambient Hg to added Hg. The increase in MeHg and THg, a ratio of 0.28:17, or 0.02 (ΔMeHg:ΔTHg), indicates that when added at low concentrations mercury from Bear Creek was 150% more available than Hg^{2+}. Taken together, these results suggest that net production of MeHg in response to THg is not linear near these *in situ* concentrations. Results from additions at ambient or near-ambient concentrations of THg are, however, more environmentally relevant than those using higher concentrations of THg. Disproportionate availability of Hg^{2+} and non-linear

behavior (both with respect to concentration and season) combined has large implications for management of mercury loads to the estuary: small loads of THg may be more significant (depending on form) than large loads especially during an ecologically relevant time (increased spring/summer primary production).

In situ sediment transplant experiments were exposed to a myriad of factors that may have impacted mercury methylation including: temperature fluctuations, longer incubation times, new sediment deposition and subsequent burial, erosion, and variation of biological, hydrological, and chemical conditions in the *in situ* environment. These *in situ* experiments closely mimicked natural depositional events, and in spite of complexities and potentially confounding affects, the resultant methylation reflected all processes acting to control it.

Net production of MeHg was greater in all treatments amended with Hg^{2+} and methylation efficiency followed the order of Hg^{2+} > Starr Tunnel (Sierra) > Bear Creek (coast). Thus results from field tests are consistent with laboratory findings and provide further evidence that within the Delta both concentration and form control MeHg production. Sediment depth appears to play a critical role in the methylation of varying forms of mercury. Lower net MeHg production in treatments amended with Hg from Bear Creek and Star Tunnel and higher net MeHg production in Hg^{2+} amended treatments suggest that *in situ* methylation at depth may be controlled more by form of Hg than concentration.

Methylation efficiency decreased with depth, consistent with previous studies (Choe et al. 2003). This is most likely a function of increasing sulfide with depth

observed in Delta sediment by others (Gill 2002). This finding is consistent with HgS as the dominant form available for methylation in sulfidic pore waters (Benoit 1999, Jay et al. 2002). Burial and resuspension of sediment is occurring within the Delta during the summer (due to tidal currents) and thus during a critical period with respect to methylation. Consequently, the MeHg problem in the Delta may be a function of resuspension and burial not only during storm events but also throughout the seasonal cycle.

If Hg entering the system during the winter is buried, and remains buried, it may contribute less to the overall MeHg budget for the estuary than ongoing sources during non-storm periods. In addition, burial may provide an environment where sulfide can bind to Hg and form the less available form HgS. It is possible that the lower methylation efficiency observed in the fall is in part due to species change during early diagenesis. During the fall new sediment to depositional habitats is from sources other than incoming riverine sediment loads like those associated with high flow events during the winter. Sources of sediment during the summer and fall include agriculture irrigation returns and that which re-distributed during flood- ebb cycles like that observe in the macrotidal Gironde Estuary (Tseng et al. 2001). Thus, the surficial sediment tested in this study during the fall was likely recently scoured from a more distant location and re-deposited to where the tests were conducted. If the sediment had been recently buried to depths at which sulfide should be increased (e.g. >1 cm, Gill et al. 2002), any available Hg should have been converted to HgS. Due to constant reworking of sediments in the Delta by tidal processes over the summer, it is likely that Hg does not remain buried, and

as such should contain Hg in the form of HgS regardless of the current biogeochemical environment in which it is found. Thus, during the summer/fall the source of more readily available Hg should not be mercury contained in sediments from wintertime flows, but rather a different source such as new mercury from sources during the summer, or deposition of organic matter containing mercury (not previously buried). These new, ongoing sources of Hg may be more relevant with respect to the MeHg problem if they are an efficiently methylated form, they arrive during time periods when bioavailable forms may be more readily methylated (e.g. spring/summer), and continuously deposit to near surface sediments where biogeochemical conditions are optimal for methylation and methylmercury flux to the water column.

Greater methylation efficiency in the field tests versus the lab tests for Franks Tract (all treatments) suggest that in some cases lab results may underestimate methylation in the field. Sulfide inhibition in the lab is not likely the source of this discrepancy because Hg^{2+} was methylated with greater efficiency than other forms suggesting lack of sulfide. The far greater methylation efficiency in the Hg^{2+} treatment in the field (6.5 times greater than in the lab) suggests that laboratory tests may severely underestimate *in situ* MeHg production in surficial sediment. Another mechanism that may help explain these results is the phenomena of tidal flushing (Caetano et al. 1997), lacking in the lab experiment. If tidal flushing (combined with or enhancing bioturbation) provides a mechanism that flushes a portion of the sulfide out of the surficial sediment, then it is possible that, where conditions exist, lack of sulfide inhibition could be occurring during the fall despite greater sulfate reduction and build-up

of sulfate from summer to fall. Microenvironments that contain oxygen and sulfide gradients optimal for methylation may exist within sediment with greater microbial activity due to a combination of factors including bioturbation and tidal plumping.

Where burial occurred (Cache Slough and 14 Mile Slough), the lab tests appear to overestimate the predicted methylation that is actually occurring in the natural environment. Methylation efficiency in controls did not change with depth at Cache Slough, suggesting HgS (or similarly less available form) was the dominant form methylated in the surficial sediment. Conversely, methylation efficiency in deep Hg^{2+} treatments decreased to even lower than observed in controls from the same depth. Methylation efficiency appears to be related to additional factors in this case. A plausible explanation for the lower methylation efficiencies is that the added Hg^{2+} was bound to other constituents than sulfur (similar to that found by other researchers; Kim et al. 2004, Slowey et al. 2005). Ionic mercury may have bound with mineral clays (a strongly complexed mineral lattice form; Bloom 2002). This would make sense if in the absence of organic matter (lower LOI and thus less humic and fulvic acids available to bind with sulfur groups) both sulfur and other groups combine to limit methylation.

The discrepancy between sites with burial may also be explained by a loss of MeHg in the Hg^{2+} amended treatment at depth at 14 Mile Slough, thus suggesting an increased loss factor related to depth and organic matter. During early diagenesis methylmercury may bound with dissolved organic carbon and migrate out of the sediment with porewater traveling upward as new weight of added sediment squeezes the

sediment. This is consistent with field observations that sediment at depth was more consolidated and appeared to contain less pore water than shallow sediments.

The observed loss of experimental mercury from both the Hg^{2+} and Starr Tunnel plots by week eleven (Franks Tract) suggest the entire mass of amended mercury may have been methylated and exported to the environment. Furthermore, evidence from Franks Tract indicates that MeHg produced *in situ* may be exported to the environment at rates near what the proxy (MeHg:THg) predicts. The amended Hg may have been more labile in the MeHg form and therefore subject to loss via bioirrigation, tidal pumping (sediment-water exchange), and other loss factors. A net methylation rate of 7 ng/g per day (0.01 methylation efficiency) would be needed to explain this phenomenon, a rate well within observed instantaneous rates (this study; Heim 2003, Marvin-DiPasquale and Agee 2003). Yet, this rate is over two orders of magnitude greater than previous MeHg flux measurements from Franks Tract (10 ng/m^2 per day; Gill 2002). Conversely, the amended Hg may have been dispersed in its original form (THg) by bioturbation, tidal pumping, or scour. Removal in the THg form may be more plausible because previously reported THg flux from Franks Tract sediment (150 ng/m^2 per day) is greater than reported MeHg flux (Gill 2002). Nevertheless, loss of either organic or inorganic *in situ* Hg at this rate has large implications with respect to the cycling of Hg in the environment. Loss of *in situ* amended Hg was significantly less at Cache Slough and 14 Mile Slough, suggesting that burial minimizes Hg sediment-water exchange and thus preserves THg.

This study indicates that buried mercury is preserved in a form less available for methylation. However, this effect may be mitigated when greater organic matter is present (dissolved organic matter, humic and fulvic acids). Thus, burial of sediment high in mercury may be used as a way to reduce methylmercury exposure only after we understand the connection with organic matter, pore water, and early diagenesis. This is particularly relevant to dredging, levee work, and use of dredged materials within the Delta.

These findings help substantiate previous findings that Delta regions with most highly elevated biotic Hg were dominated by ongoing new inflows of Hg from upstream sources (Slotten et al. 2002), and that mineral-derived Hg (coast range) is converted to MeHg less efficiently that forms from the Sierras (Bloom 2002, Suchanek et al. 2002). Several researchers have used anoxic slurries and non-native sediments to evaluate sediment methylation potentials from a number of areas (Bloom 2002, Slotten et al. 2002, Suchanek et al. 2002, Marvin-DiPasquale and Agee 2003). Using whole intact sediment cores and *in situ* dosing experiments, the experimental results obtained here, although complex, are perhaps more ecologically relevant. Readily available mercury was easily and rapidly methylated (within 1 day) once incorporated into Delta sediments (results, this study), a phenomenon also observed by Slotten et al. (2-8 days; 2002). Elemental mercury (American River) was the most available form of mercury from the environment tested (90% relative to Hg^{2+}; Franks Tract). On average, forms of mercury in Bear Creek and Starr Tunnel sediment were less available, about 5% and 10%, respectively. Thus, results suggest that while a decrease in Hg loads from upstream sources on either side of

the watershed should decrease net MeHg production in Delta sediments, it is a decrease in loads of Hg^0 like that found in the American River that would result in the greatest reduction. This, and the nearly 1,000 times greater Hg concentrations found in the American River, make waterbodies contaminated with Hg^0 from past gold mining activities primary candidates for mitigation. Available forms of mercury may, however, be converted to cinnabar, thus it is not clear if available forms of Hg from the Sierra actually enter the Delta. Although it is likely that available forms of Hg are more relevant during portions of the year not characterized by mass loading of Hg from high flow storm events, the relatively greater magnitude of Hg entering the Delta from coastal and Sierra sources are important considering that regardless of form, increased inorganic Hg leads to increased MeHg.

Further, 97% of the total mercury load (215 kg/yr) to the Delta comes from tributary inputs (Sacramento-San Joaquin Delta Estuary TMDL for total and methylmercury 2005). Results indicate that mercury from the Sacramento River and San Joaquin River (contaminated with Hg^0 from the Sierras and delivering 149 kg/yr and 19 kg/yr of THg, respectively) may be the predominant source of mercury to methylating habitats. Thus, a reduction in loads from these sources may have the greatest impact. Conversely, mitigation of loads from Prospect Slough (36 kg/yr) may have less impact towards reduction of net MeHg production in the Delta due to the relatively reduced methylation efficiency of mineral-derived Hg in sediment from this watershed (results this study). If mercury in the environment is converted to HgS during erosion, transport and subsequent burial, the relative contribution of historic mercury to the MeHg problem

today is unclear. Although loads of Hg^{2+} via direct and indirect wet deposition (~1%) are relatively small, the disproportionately greater availability of this form suggests that atmospherically derived Hg may be important with respect to MeHg production in the Delta. Results from Franks Tract implicate another source of available Hg in the fall; Suisun Bay sediment containing elevated concentrations of Hg (ascribed to gold tailings) of up to 950 ng/g at a depth of 30 cm (Hornberger et al. 1999) may be eroding and migrating within the estuary. This erosion and migration may be responsible for the observed increase in water column inorganic Hg at mid-bay salinity ($12^{o/oo}$ salinity; Gill 2002).

Conclusions

This study demonstrates that while many factors control the production of MeHg, chemical form and concentration of the Hg are among the most important. Additions of mercury contaminated sediment from upper watershed sources increased net methylmercury production when mixed and transplanted into Delta sediment at all three locations: Franks Tract, Cache Slough, and 14 Mile Slough. These findings are especially relevant because the wetland habitat studied is found widespread in the Delta. Findings also indicate that net MeHg increased with increasing THg dose with high degree of statistical confidence, and that this relationship is linear in some cases over 10,000 ng/g THg. This local evidence suggests that reduction of THg to the Delta should decrease MeHg production in sediments and thus also biotic MeHg exposure. This is especially relevant in the case where other factors controlling methylation efficiency

cannot be controlled because regardless of all other factors, amount of THg controls amount of MeHg.

Total mercury generally has point sources (mines, reservoirs, permitted discharge, or identified source watersheds), whereas biogeochemical factors (organic carbon, sulfate, redox, sedimentation rate) have non-point sources and are difficult to control. Mitigation of MeHg thus begins with mitigation of THg at the origin. Point sources of THg are therefore prime targets for mitigation because they are more easily controlled than other factors, and if transported off-site, this mercury has a propensity to enhance MeHg production in habitat found widespread in the Delta ecosystem.

To control production of methylmercury in sediments, reduction of inputs of elemental mercury from the Sierra should be the highest priority. Locations with elemental mercury like that found in the American River should be considered prime candidates for mitigation due to greater environmental concentrations of Hg and availability for methylation. Mercury that is sorbed with clays (like that from the Starr Tunnel) may be of secondary priority for mitigation due to decreased methylation efficiency. Sediment from the coast range (HgS) was the least efficiently converted to MeHg, indicating that mitigation of these sediments may be lower priority. However, it is important to realize that preferentially mitigating certain forms of Hg may make the greatest difference only immediately downstream. In other words, if mercury is converted to HgS when in sulfidic environments, it is likely that this ranking system may change. In this scenario, ongoing new sources of available mercury become more important for mitigation.

This study indicates that the relationship between MeHg and THg was habitat specific and methylation efficiency was highly variable between sites. Future mercury mitigation strategies could be designed to mimic conditions found at Cache Slough where at times very little MeHg was produced and methylation efficiency was relatively low. Future work needs to address the factors controlling differences in methylation efficiency between sites. Understanding these factors will supply needed information to water resource managers in cases where we do have the ability to manipulate habitats (e.g. restored and constructed wetlands) or manage the other controlling factors once determined.

This work indicates that varying forms of Hg found in the environment are methylated with varying efficiencies. At a particular location, increased methylation efficiency observed is a function of the form of mercury available for methylation. Although the sulfur cycle plays a role in this, there first has to be an available form present to allow for increased efficiency. The greater availability of Hg^{2+} for methylation during the summer suggests that the MeHg in the system is controlled in part by new sources of available mercury during this critical time of enhanced primary productivity. This study suggests that Hg speciation may be a contributing factor in observed seasonality of net MeHg production in Delta sediments. Speciation as a contributing mechanism needs further study to enhance understanding its role relative to other mechanisms.

Results of *in situ* sediment transplant experiments give greater support to the above findings because they integrate all processes acting on mercury methylation.

Processes controlling methylation in wetland habitats is complex requiring *in situ* approaches that integrate the *in situ* complexity of these systems, thus experiments using this approach should be the method of choice. Methods described in the work for the laboratory whole-core incubations mimic the natural environment and are thus inherently better for conducting experiments with the goal of understanding natural phenomena. Overall, these methods are a simple, cost effective way of determining the relative risk of methylmercury production in sediments. These methods could easily be used for a variety of experiments designed to help understand methylation: for instance, 1) measure the availability for methylation of THg from a variety of sources, 2) measure the efficiency of methylation in sediment from proposed wetland restoration sites, and 3) measure the effect of other factors on methylation efficiency (i.e. organic carbon, sulfate, sulfide, salinity, and others). Because the laboratory-based tests have been shown to maintain critical biogeochemical gradients, they could provide methodology for sediment bioaccumulation and toxicity tests such that these types of tests be conducted using substrate with environmental parameters representative of those found in the natural environment.

In this study, processes that were previously underestimated and poorly understood with respect to the MeHg problem in the Delta were found to be very relevant with respect to methylation. In particular, sediment within the Delta is extremely mobile; it is eroded, suspended, and deposited to locations potentially both near and far. This phenomenon allows for continual change of biogeochemical conditions and thus also the constant reestablishing of specific conditions that enhance MeHg production. This

process has profound implications for the cycling of Hg in the environment. Mercury methylation in the Delta is part of a system in which Hg contaminated particles are constantly redistributed within and among basins of various sizes. Burial, and thus post-depositional environment and early diagenesis, are significant *in situ* factors that must be understood with relation to methylation, fluid transport of Hg, and Hg speciation. Mercury that is buried converted to a less available form, suggesting that through the process of transport from upper watershed sources to the Delta mercury of a variety of forms may be eventually all converted to cinnabar. Regardless, once within the estuary, mercury is processed through resuspension and reburial in the estuarine environment and exposed to greater sulfide concentrations found in this sediment. This likely enhances the transformation to HgS. Furthermore, processes occurring during transport of sediment (such as reoxidation and exposure to sulfate and a variety of Hg species) may be of significant importance to understanding MeHg production in sediment.

Scientists and regulators should use caution when attributing environmental cause and effects of *in situ* sediment MeHg production to local conditions because this sediment, or characteristics of this sediment, may not have a local origin. Future studies need to investigate methods for the evaluation of MeHg production in sediments under conditions occurring during scour, suspension, and deposition, and towards the understanding of MeHg production with regards to frequency, duration, and depth of inundation (mechanisms that resource managers can control).

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Table 1. Meta data for source sediment.

Site	Collection Date	Watershed	Environmental Location of Hg	Downstream Methylating Habitat	Hg Description	THg ng/g
American River	Summer 2003	Sierras: Sacramento	In-Stream	Folsom Reservoir, North East Delta, SF Bay-Delta	Elemental	25,000,000
Starr Tunnel	14 July 2004	Sierras: Yuba River	Rivulet within sluice tunnel discharging to stream	Rollins Reservoir, North Delta, SF Bay-Delta	Elemental - clay matrix	33,000
Bear Creek	9 August 2003 14 July 2004	Coast Range: Cache Creek	In-Stream	Yolo Bypass, North Delta, SF Bay-Delta	Cinnabar & geothermal	2,500

Table 2. Site selection criteria for sampling locations in the Delta: Franks Tract, Cache Slough, and 14 Mile Slough.
*Denotes that data is from CALFED Mercury Project (Heim 2003).

Site	Area	Primary Water Source	Primary Hg Source	THg (ng/g)*	MeHg (ng/g)*	MeHg: THg*	Loss on Ignition (%)*
Franks Tract	Central Delta	Mixed: Sacramento River, San Joaquin River and SF Bay	Mixed: Riverine (coast range and Sierras) and Bay (e.g. re-suspended sediment from Suisun Bay)	165	1.69	0.009	9.8
Cache Slough	North Delta	Sacramento River (chloride dominated)	Coast Range: Cache Creek watershed	124	0.44	0.003	2.9
14 Mile Slough	South Delta	San Joaquin River (sulfate dominated)	Sierras: Mokulmne River and San Joaquin River watersheds	138	2.99	0.02	13.3

Table 3. Abbreviated procedure for conducting methylation experiments using laboratory methods (intact incubated whole sediment cores).

Whole-Core Dosing Experiments	
1 Collection	a) Collect intact sediment cores and surficial sediment from receiving sediment locations (Delta sites). b) Collect source sediment from locations upstream of Delta known to contain elevated mercury (in-stream sediment contaminated with mine waste).
2 Laboratory Storage	a) Equilibrate cores to laboratory conditions. b) Store surficial sediment from receiving sediment locations and source sediment at refrigerator temp.
3 Preparation	Mix source sediment with surficial receiving sediment to form slurry and achieve target Hg concentrations desired to test.
4 Test initiation	Mimicking a natural fluvial deposition event, deposit 1 cm of Hg containing slurry to surface of core.
5 Incubation	Eight days with constant aeration and temperature (20° C).
6 Test termination	Remove the portion of Hg containing sediment that was added during test initiation (slice off the top 1 cm), place in storage container, and freeze.
7 Analyses	Analyze samples for THg and MeHg.

Table 4. Abbreviated procedure for conducting methylation experiments using field methods (*in situ* sediment transplants).

***In-situ* Sediment Transplant Experiments**		
1	Collection	a) Collect surficial sediment from receiving sediment locations (Delta sites). b) Collect source sediment from locations upstream of Delta known to contain elevated mercury (in-stream sediment contaminated with mine waste).
2	Laboratory Storage	Store surficial sediment from receiving sediment locations and source sediment at refrigerator temperatures.
3	Preparation	Mix source sediment with surficial receiving sediment to form slurry and achieve target Hg concentrations desired to test. Analyze for THg and adjust accordingly.
4	Test initiation	Identify benthic plot and place plastic enclosure to isolate water column above the plot. Mark surface of benthic plot with thin layer of inert white sand. Mimicking a natural fluvial deposition event, deposit 1 cm of Hg containing slurry to surface of plot. Allowing sediment to settle, remove enclosure after 24 hours.
5	Incubation	Days to months.
6	Test termination	Core the sediment in the plot and sample the portion of Hg containing sediment that was added during test initiation (slice multiple 0.5 cm sections near the marker sand), place in storage container, and freeze.
7	Analyses	Analyze samples for THg. Identify the sample (core section) with the added THg, and analyze for MeHg.

Table 5. Environmental data collected at the three locations in the Delta during this study: Franks Tract, Cache Slough, and 14 Mile Slough. ND = not determined.

Site	Date	pH	EC ($\mu S/cm^2$)	DO (%)	Water Temp (°C)	Sediment Temp (°C)
Frank's Tract 38.05378° N 121.59003° W 3.0 m water depth	11-5-03	7.63	449	91	15.6	17.1
	11-18-03	7.05	515	97	14.2	14.2
	6-16-04	9.75	357	180	23.4	24.4
	7-6-04	7.71	272	91	22.7	24.0
	7-14-04	ND	ND	ND	ND	24.3
	7-19-04	8.10	ND	ND	24.6	24.1
	8-7-04	8.70	290	107	23.1	22.7
	9-25-04	ND	550	88	20.5	21.7
Cache Slough 38.28624° N 121.71856° W 3.0 m water depth	11-18-03	8.37	185	ND	13.2	13.1
	6-17-03	ND	ND	ND	ND	21.3
	7-6-04	6.98	194	92	20.8	22.3
	7-15-04	7.80	252	85	25.1	23.5
	7-21-04	7.81	190	88	26.2	22.9
	8-7-04	8.43	191	120	25.3	21.7
	9-25-04	ND	235	110	22.0	19.1
14Mile Slough 38.00410° N 121.40275° W 2.5 m water depth	11-18-03	7.12	530	99	14.6	14.7
	6-17-03	ND	ND	ND	ND	25.5
	7-6-04	7.82	240	99	26.8	27.0
	7-15-04	7.80	425	110	27.1	25.9
	7-21-04	7.34	407	91	26.9	25.7
	8-8-04	7.46	483	60	25.1	25.1
	9-26-04	ND	645	110	22.7	21.1

Table 6. Loss on Ignition and size distribution of sediment particles (by volume) for each sampling location in the Delta: Not Treated (NT) and treated with hydrogen peroxide (T).

Site	Event	Loss On Ignition (%)	Clay % < 4 um		Silt % 4-63 um		VF Sand % 63-125 um		F Sand % 125-250 um		M&C Sand % 250-2000 um	
			NT	T	NT	T	NT	T	NT	T	NT	T
Cache Slough	Fall 2003	5.7	20.2	30.3	70.5	66.3	6.5	2.8	2.4	0.6	0.4	0
	Summer 2004	6.1	25.0	40.3	68.8	59.6	4.1	0.2	2.0	0	0.1	0
Franks Tract	Fall 2003	8.7	12.1	28.2	68.4	64.2	8.0	4.5	6.3	3.0	5.1	0
	Summer 2004	7.1	21.2	35.3	62.6	59.6	8.9	3.9	6.5	1.3	0.8	0
14 Mile Slough	Fall 2003	8.0	3.6	9.3	24.8	32.5	24.1	27.9	19.7	17.9	27.7	12.5
	Summer 2004	13.5	7.5	18.3	38.7	51.6	13.4	16.2	14.5	9.1	25.7	4.8

Table 7. Methylation efficiency experiments conducted during the fall (2003) using intact sediment cores from Franks Tract. THg and MeHg (ng/g dw), and MeHg:THg ratios at two depths, 0-1 and 1-2 cm (days 0, 1, 2, 4, and 8). Treatments: 1) control (slurry) = method control (addition of sediment slurry, no Hg dose), 2) dosed with Hg^{2+}, and 3) amended with sediment containing Hg from Bear Creek.

	Day	Control (slurry)		Bear Creek		Hg^{2+}	
		0-1 cm	1-2 cm	0-1 cm	1-2 cm	0-1 cm	1-2 cm
THg	0	130	152	146	NA	396	NA
	1	143	146	243	NA	449	NA
	2	139	146	143	147	542	188
	4	148	153	171	149	467	251
	8	125	134	179	133	448	159
	Ave. ± SD	137 ± 9.4	146 ± 7.6	176 ± 40.3	143 ± 8.7	460 ± 52.8	199 ± 47.0
MeHg	0	1.76	0.97	1.85	NA	1.75	NA
	1	1.97	1.00	1.49	NA	20.40	NA
	2	1.73	2.38	2.28	1.93	28.82	5.14
	4	2.28	1.82	1.74	2.29	20.89	6.14
	8	1.42	1.24	1.82	1.62	28.86	3.35
	Ave. ± SD	1.85± 0.37	1.61 ± 0.62	1.83 ± 0.33	1.95 ± 0.34	24.74 ± 4.74	4.97 ± 1.54
MeHg:THg	0	0.0135	0.0064	0.0126	NA	0.0044	NA
	1	0.0138	0.0068	0.0061	NA	0.0454	NA
	2	0.0124	0.0163	0.0159	0.0131	0.0532	0.0273
	4	0.0154	0.0119	0.0102	0.0154	0.0447	0.0256
	8	0.0113	0.0092	0.0102	0.0122	0.0644	0.0211
	Ave. ± SD	0.0133 ± 0.0015	0.0101 ± 0.0041	0.0106 ± 0.0040	0.0136 ± 0.0016	0.0519 ± 0.0092	0.0246 ± 0.0032

Table 8. Methylation efficiency experiments conducted during the fall (2003) using intact sediment cores from Delta locations Franks Tract, Cache Slough, and 14 Mile Slough. Mean THg and net production of MeHg (values in ng/g dry weight). MeHg:THg in sediment from controls, three concentrations of amended Hg^{2+} [2 (2X), 4 (4X), and 8 (8X) times ambient concentration], and amended with mine-derived sediment from Bear Creek or American River (20 °C; n=3, ± SD).

	Location	Control (no slurry)	Control (slurry)	Bear Creek	American River	Hg^{2+} 2X	Hg^{2+} 4X	Hg^{2+} 8X
THg	Franks Tract	168 ± 40.6	154 ± 10.7	167 ± 14.2	10700 ± 1320	231 ± 25.1	414 ± 17.6	686 ± 9.02
	Cache Slough	103 ± 14.6	102 ± 3.6	115 ± 8.1	7530 ± 968	184 ± 8.7	390 ± 30.5	725 ± 48.5
	14 Mile Slough	145 ± 23.8	129 ± 9.6	181 ± 46.7	13100 ± 1180	213 ± 13.6	431 ± 34.5	814 ± 23.9
MeHg	Franks Tract	1.29 ± 0.51	1.29 ± 0.07	1.80 ± 0.49	137 ± 45.9	3.32 ± 0.85	6.52 ± 0.92	10.9 ± 3.0
	Cache Slough	0.25 ± 0.04	0.26 ± 0.02	0.34 ± 0.05	1.43 ± 0.28	0.54 ± 0.07	0.96 ± 0.09	1.77 ± 0.29
	14 Mile Slough	1.17 ± 0.26	0.85 ± 0.09	1.11 ± 0.11	24.4 ± 8.33	1.60 ± 0.07	3.03 ± 0.25	4.59 ± 0.62
MeHg:THg	Franks Tract	0.0081 ± 0.0045	0.0084 ± 0.0009	0.0106 ± 0.0020	0.0127 ± 0.0033	0.0142 ± 0.0022	0.0158 ± 0.0028	0.0158 ± 0.0041
	Cache Slough	0.0025 ± 0.0006	0.0026 ± 0.0001	0.0030 ± 0.0005	0.0002 ± 0.00003	0.0029 ± 0.0003	0.0025 ± 0.0003	0.0024 ± 0.0002
	14 Mile Slough	0.0081 ± 0.0009	0.0066 ± 0.0011	0.0064 ± 0.0015	0.0018 ± 0.0005	0.0075 ± 0.0007	0.0070 ± 0.0004	0.0057 ± 0.0009

Table 9. Methylation efficiency experiments conducted during the summer (2004) using bulk sediment pretreated at cold temperature (1°C) from Delta locations Franks Tract, Cache Slough, and 14 Mile Slough. Mean THg and net production of MeHg (ng/g dry weight). MeHg:THg in sediment from controls, amended with Hg^{2+}, and amended with mine-derived sediment from Bear Creek or the Starr Tunnel (n=3, ± SD).

	Location	Control (slurry)	Bear Creek	Starr Tunnel	Hg^{2+}
THg	Franks Tract	178 ± 43.1	891 ± 364	771 ± 172	791 ± 186
	Cache Slough	121 ± 39.6	928 ± 602	532 ± 96.6	623 ± 175
	14 Mile Slough	150 ± 36.0	1090 ± 101	828 ± 190	927 ± 119
MeHg	Franks Tract	0.75 ± 0.01	1.36 ± 0.03	1.06 ± .06	5.25 ± 0.47
	Cache Slough	0.72 ± 0.01	1.20 ± .01	1.44 ± .06	22.96 ± 1.01
	14 Mile Slough	1.63 ± 0.3	2.27 ± 0.13	3.38 ± 0.11	31.44 ± 1.43
MeHg:THg	Franks Tract	0.0043 ± 0.0011	0.0017 ± 0.0007	0.0014 ± 0.0002	0.0068 ± 0.0010
	Cache Slough	0.0063 ± 0.0021	0.0016 ± 0.0010	0.0027 ± 0.0004	0.0381 ± 0.0091
	14 Mile Slough	0.0112 ± 0.0025	0.0021 ± 0.0001	0.0042 ± 0.0011	0.0341 ± 0.0028

Table 10. Methylation efficiency experiments conducted during the summer (2004) using intact sediment cores from Delta locations Franks Tract, Cache Slough, and 14 Mile Slough. Mean THg and net production of MeHg (ng/g dry weight). MeHg:THg in sediment from controls, amended with Hg^{2+}, and amended with mine-derived sediment from Bear Creek or the Starr Tunnel (n=3, ± SD).

	Location	Control (no slurry)	Control (slurry)	Bear Creek	Starr Tunnel	Hg^{2+}
THg	Franks Tract	173 ± 23.0	203 ± 35.7	2720 ± 799	746 ± 203	778 ± 16.7
	Cache Slough	117 ± 9.7	129 ± 6.9	560 ± 111	476 ± 111	527 ± 26.9
	14 Mile Slough	158 ± 16.9	152 ± 4.7	1800 ± 644	790 ± 27.2	757 ± 191
MeHg	Franks Tract	0.56 ± 0.03	0.71 ± 0.06	1.26 ± 0.18	1.06 ± .09	8.74 ± 0.61
	Cache Slough	0.42 ± 0.03	0.53 ± 0.09	1.29 ± .08	1.32 ± .03	14.11 ± 2.02
	14 Mile Slough	1.15 ± 0.13	1.37 ± 0.25	2.62 ± 0.62	5.02 ± 0.61	49.58 ± 3.20
MeHg:THg	Franks Tract	0.0033 ± 0.0004	0.0035 ± 0.0003	0.0005 ± 0.0002	0.0015 ± 0.0004	0.0112 ± 0.0010
	Cache Slough	0.0036 ± 0.0004	0.0041 ± 0.0006	0.0023 ± 0.0004	0.0029 ± 0.0006	0.0268 ± 0.0040
	14 Mile Slough	0.0074 ± 0.0016	0.0091 ± 0.0020	0.0017 ± 0.0010	0.0063 ± 0.0006	0.0679 ± 0.0146

Table 11. Methylation efficiency experiments conducted during the summer (2004) using *in situ* methods: 11 week incubation period *in situ*. THg (ng/g dw) in sediments down core in half-centimeter increments from Franks Tract, 14 Mile Slough, and Cache Slough in 3 treatments: dosed with Hg^{2+}, and dosed with sediment containing Hg from Bear Creek or the Star Tunnel. * Denotes sample with amended Hg and thus analyzed for MeHg.

Depth (cm)	Franks Tract			Cache Slough			14 Mile Slough		
	Bear Creek	Starr Tunnel	Hg^{2+}	Bear Creek	Starr Tunnel	Hg^{2+}	Bear Creek	Starr Tunnel	Hg^{2+}
0.0 – 0.5	182	140	234	171	135	138	105		
0.5 – 1.0	190	159	241	171	147	139	131		
1.0 – 1.5	176	160	203	165	163	166	145		
1.5 – 2.0	255	183	195	168	186	176	145	158	138
2.0 – 2.5	1130*	184	184	387*	162	285	166		
2.5 – 3.0	220	181	159	165	200*	332*	119	144	153
3.0 – 3.5	139	196	134	161	188	334*	100		
3.5 – 4.0	184		124	162	165	231	776*	151	167
4.0 – 4.5	157		118	182	154	144	135		
4.5 – 5.0			132	155			137	149	150
5.0 – 5.5			177					236	
5.5 – 6.0								359*	136
6.0 – 6.5								204	
6.5 – 7.0								93	158
7.0 – 7.5								108	234
7.5 – 8.0								145	300*
8.0 – 8.5								142	297*
8.5 – 9.0								157	133
9.0 – 9.5								147	129
9.5 – 10									129
10 – 10.5									149
10.5 - 11									101

Table 12. Methylation efficiency experiments conducted during the summer (2004) using *in situ* methods: varying *in situ* incubation periods; 1 week, 2 week, 4 week, and 11 week. THg and MeHg (ng/g dw), and MeHg:THg ratios (± SD) in sediments of varying depths; from Franks Tract, 14 Mile Slough, and Cache Slough in 3 treatments: dosed with Hg^{2+}, and dosed with sediment containing Hg from Bear Creek or Starr Tunnel. Method controls (MC) associated each treatment of Hg dose were sampled from identical depths. Control (no slurry) = CNS.

	ID	Depth	Time	THg	MeHg	THg: MeHg
Franks Tract	MC	2 - 2.5 cm	11 week	140	0.81	0.0058
	Bear Ck			1130	1.14	0.0010
	MC	0 - 1.5 cm	1 week	64.0	1.00	0.0156
	CNS			143	0.95	0.0066
	Starr Tnl			374	0.71	0.0019
	MC	0 - 1.5 cm	2 week	122	0.41	0.0033
	Hg^{2+}			289	20.83	0.0720
Cache Slough	MC	2 - 2.5 cm	11 week	141	0.79	0.0056
	Bear Ck			387	0.14	0.0004
	MC	2.5 - 3 cm	11 week	145	0.72	0.0050
	Starr Tnl			200	0.72	0.0036
	MC	3 - 3.5 cm	11 week	120	0.71	0.0059
	Hg^{2+}			334	1.55	0.0046
	Hg^{2+}	2.5 - 3 cm		332	1.53	0.0046
	Hg^{2+}	0 - 1.5 cm	2 week	327	3.86	0.0118
14 Mile Slough	MC	3 - 4 cm	11 week	144	1.27	0.0089
	Bear Crk	3.5 - 4 cm		776	1.28	0.0016
	MC	5.5 - 6 cm	11 week	83	0.38	0.0046
	Starr Tnl			359	0.97	0.0027
	MC	7.5 - 8 cm	11 week	156	0.42	0.0027
	Hg^{2+}			300	1.92	0.0064
	Hg^{2+}	8 - 8.5 cm		297	1.37	0.0046
	Hg^{2+}	2.5 - 3.5 cm	4 week	404	5.08	0.0126

Figure 1. Gold and mercury mines in California (Alpers and Hunerlach 2000), locations of source sediment collection for this study, and the location of the Sacramento – San Joaquin Delta within California.

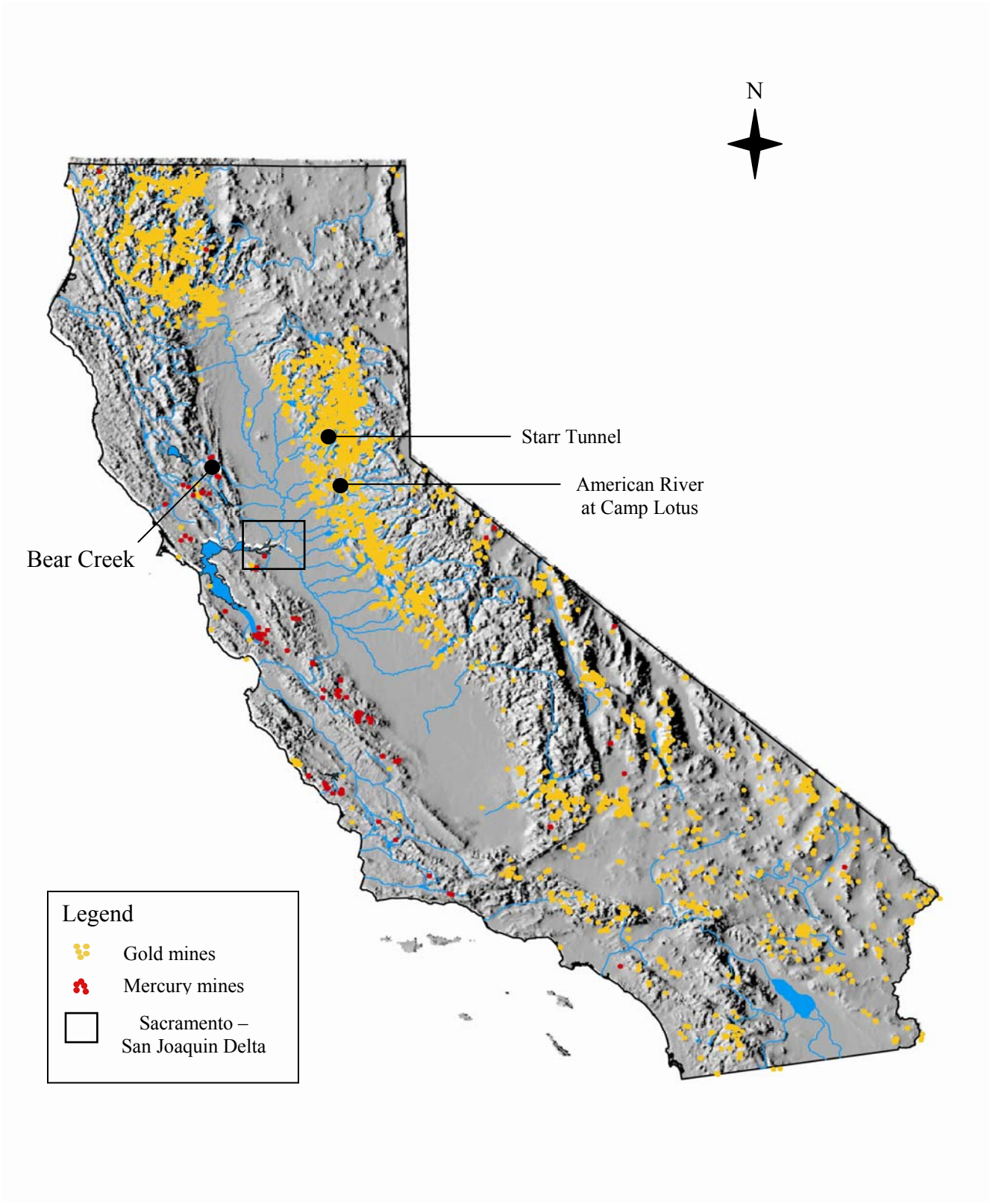


Figure 2. Receiving sediment sampling locations in the Sacramento - San Joaquin Delta

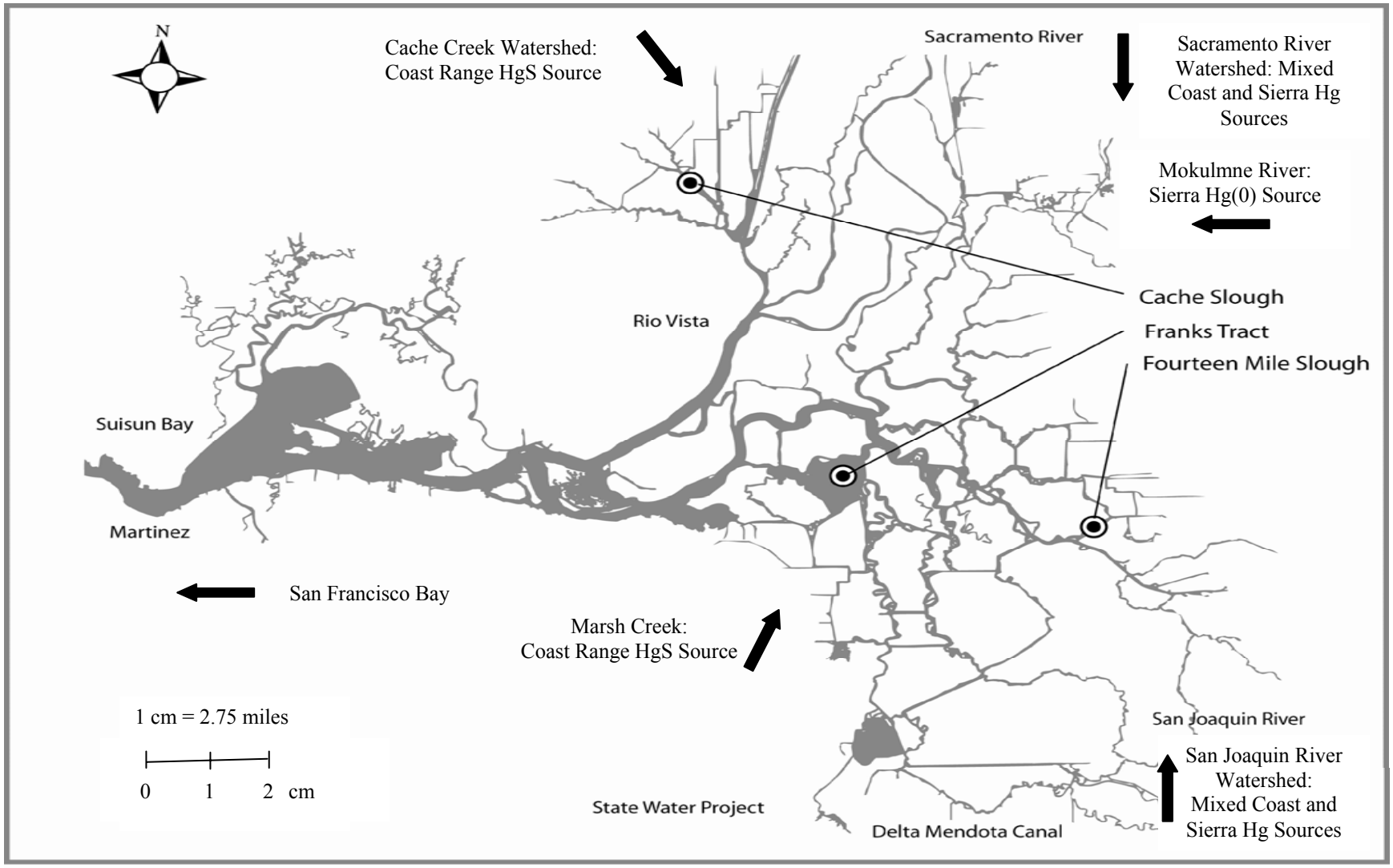


Figure 3. *In situ* sediment transplant field experimental design.

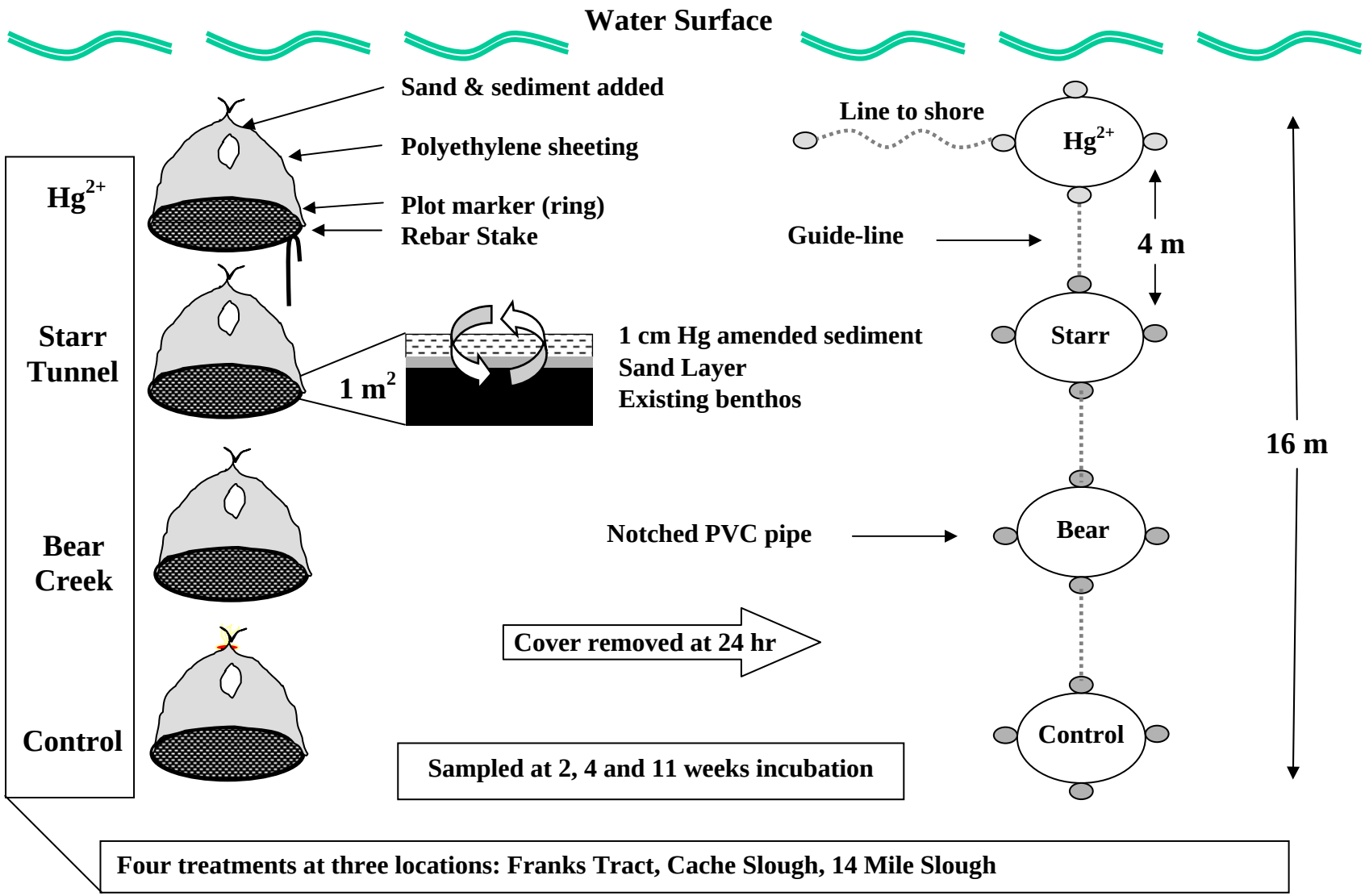


Figure 4. Size distribution of sediment particles: Cache Slough, Franks Tract and 14 Mile Slough non-treated (Bulk) and treated with hydrogen peroxide (HP; laboratory experiments conducted Summer 2004).

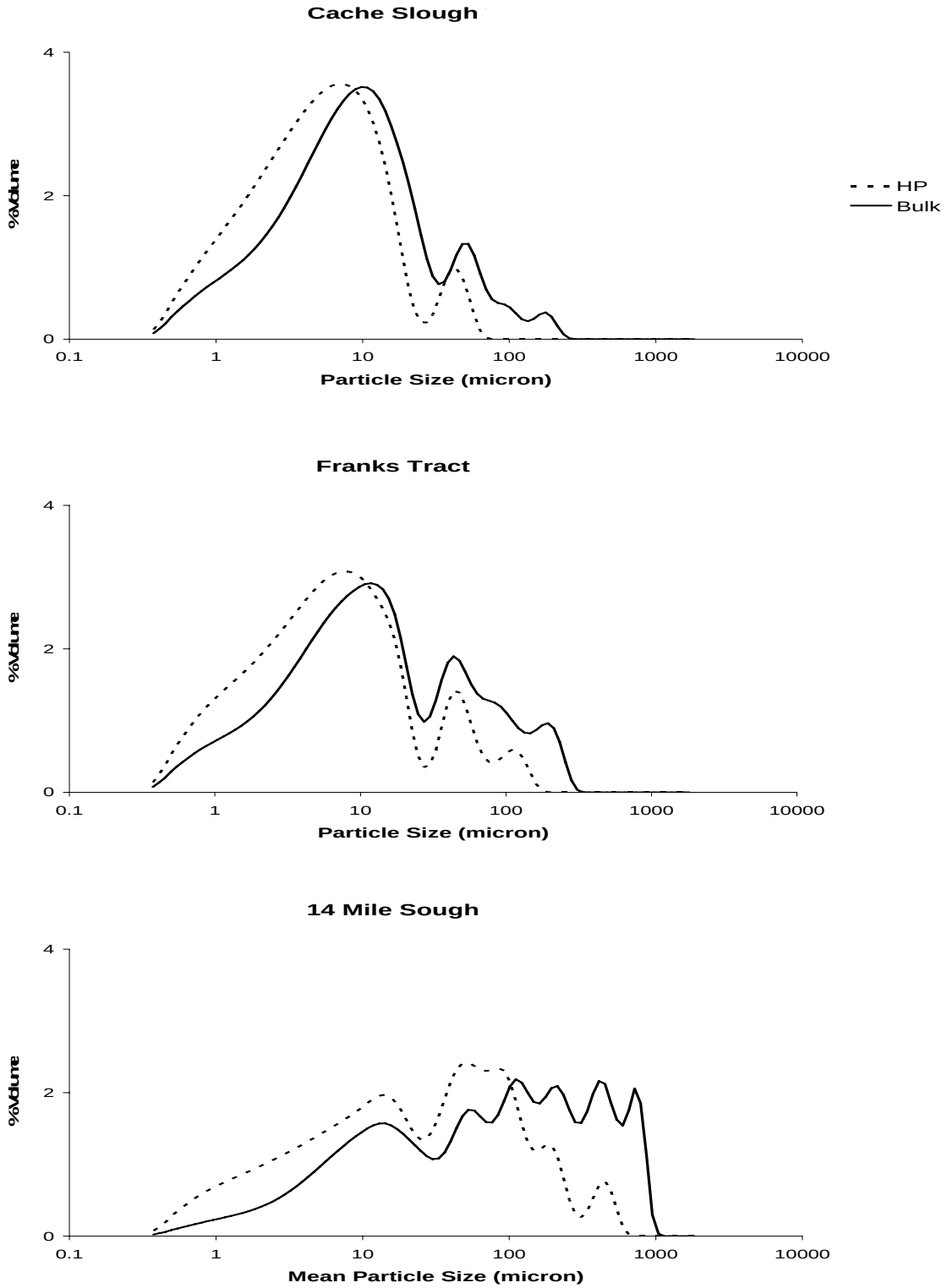


Figure 5. Net MeHg production and MeHg:THg over time in control and Hg^{2+} amended treatments (laboratory methylation experiments conducted Fall 2003 using Franks Tract sediment).

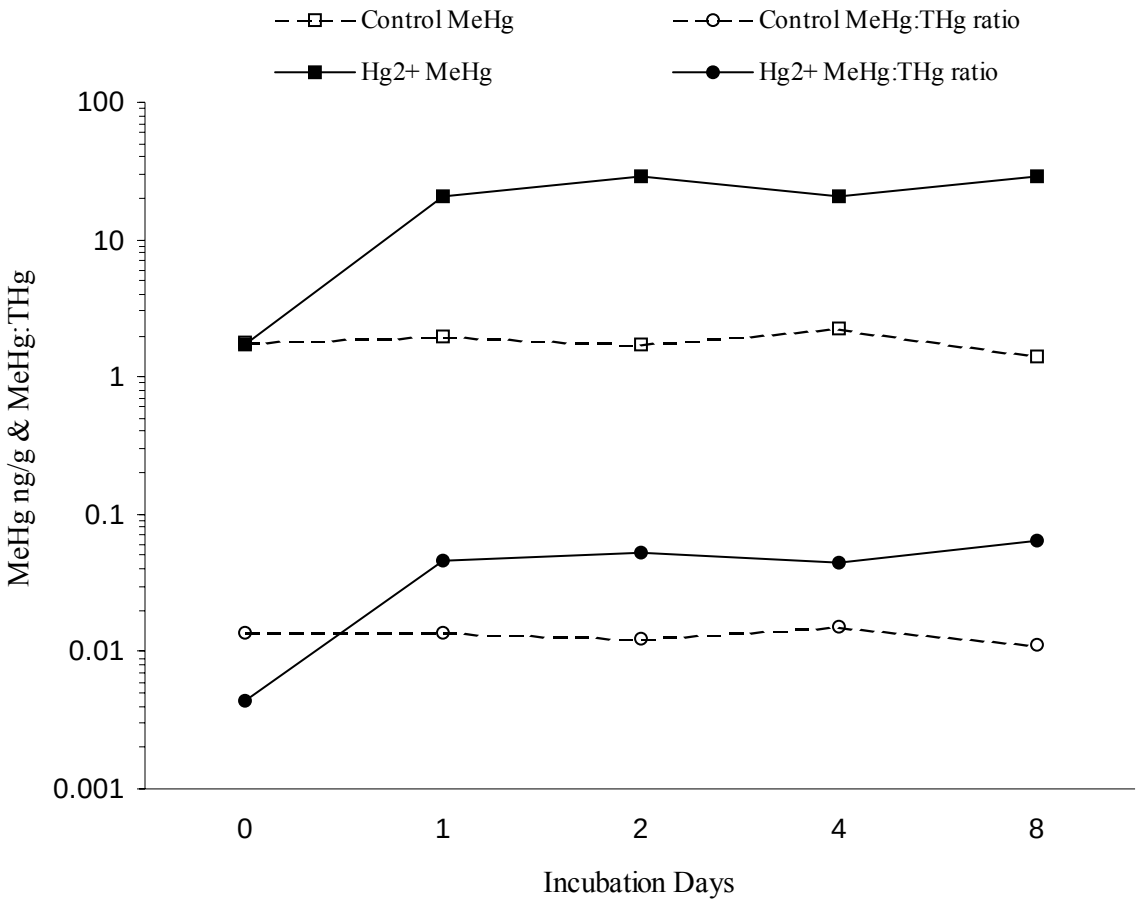


Figure 6. Net MeHg production in controls and Hg^{2+} amended treatments for each Delta location with corresponding regression lines and r-squared values (slope = MeHg:THg & methylation efficiency; laboratory methylation experiments conducted Fall 2003).

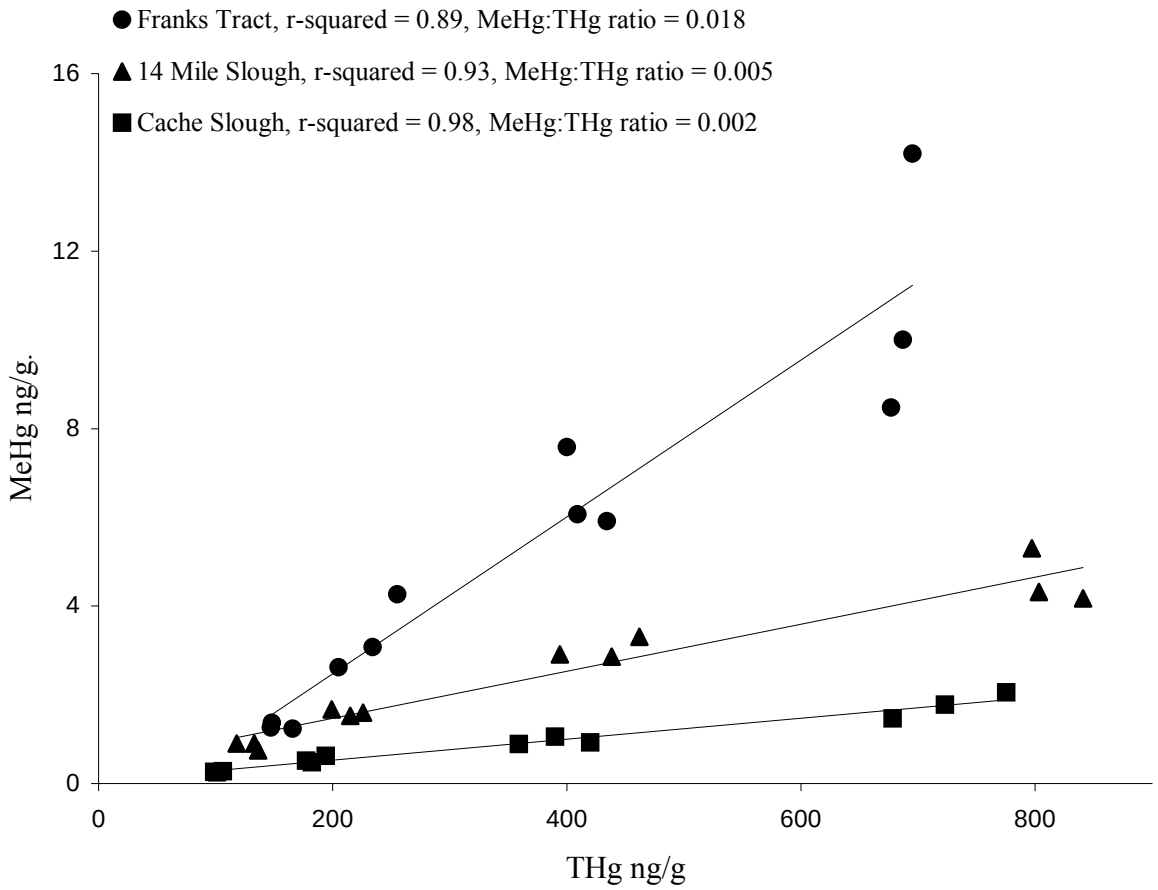


Figure 7. Methylation efficiency (MeHg:THg) in control and Hg^{2+} amended treatments for each Delta location: Franks Tract (FT), Cache Slough (CS), and 14 Mile Slough (14 MS); from laboratory experiments conducted Summer 2004 and Fall 2003.

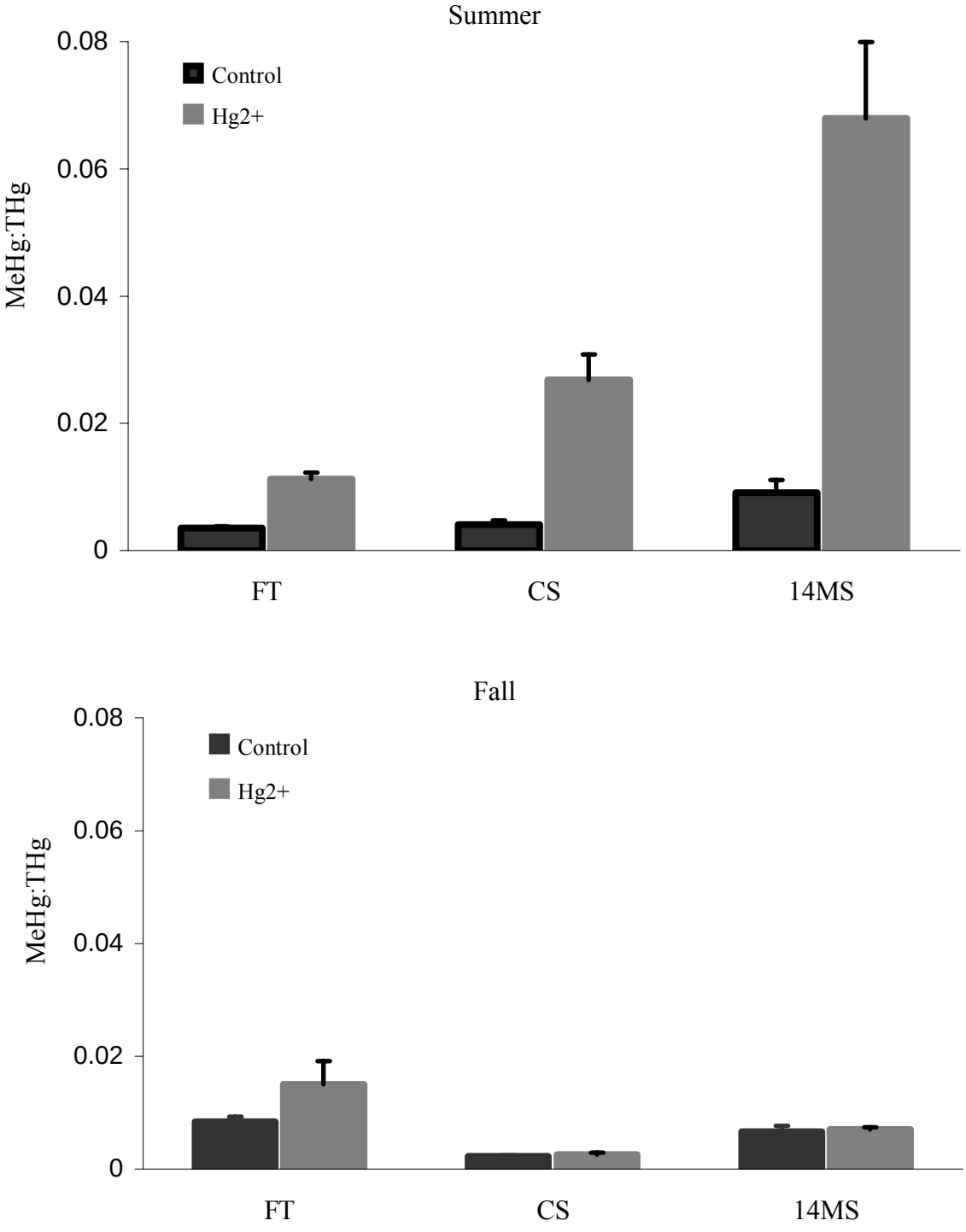
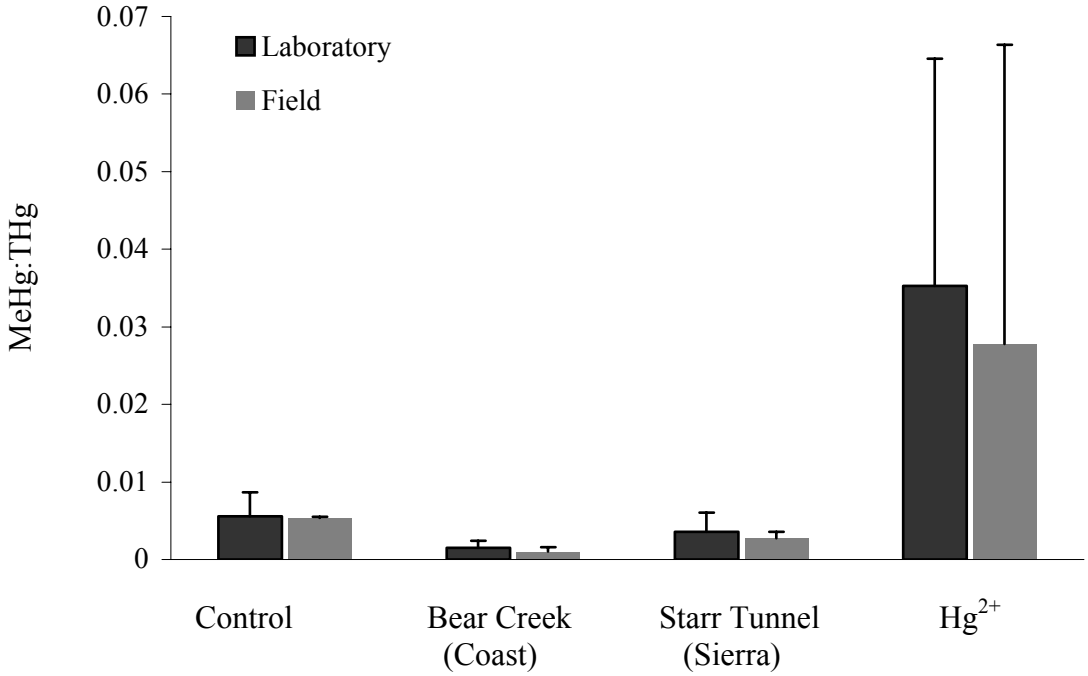


Figure 8. Average MeHg:THg (methylation efficiency) in control and Hg amended treatments from a) laboratory and field experiments conducted in the summer (2004; error bars = SD, all three Delta locations), and b) laboratory experiments conducted in the fall (2003, error bars = SD, Franks Tract).

a) Summer



b) Fall

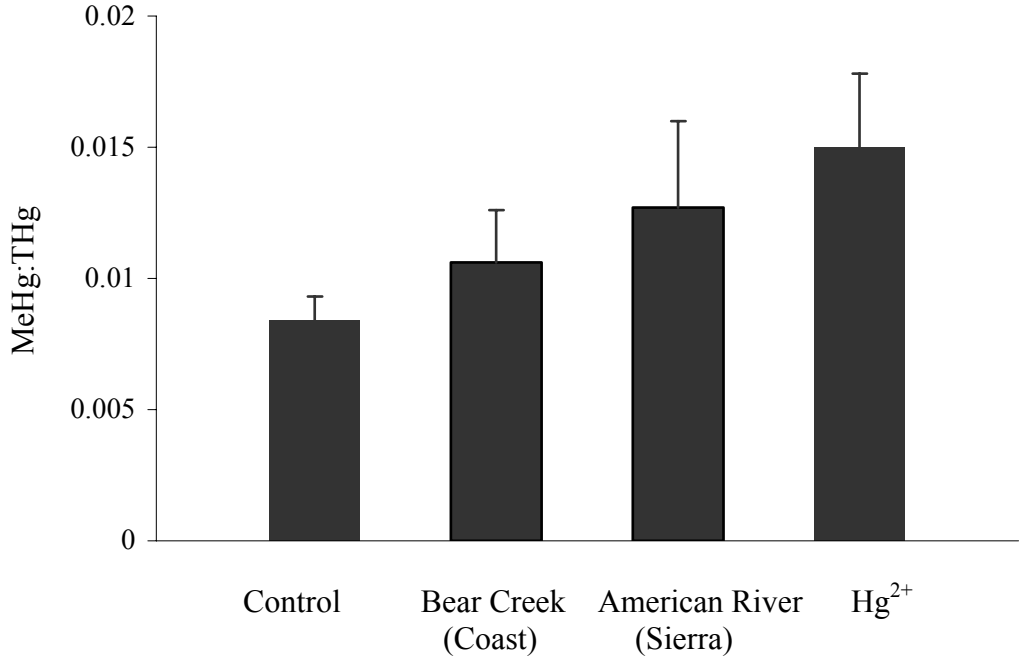


Figure 9. Net MeHg production in Control and American River amended treatments (a), and MeHg:THg (methylation efficiency) in Control and American River amended treatments (b) from laboratory experiments conducted Fall 2003.

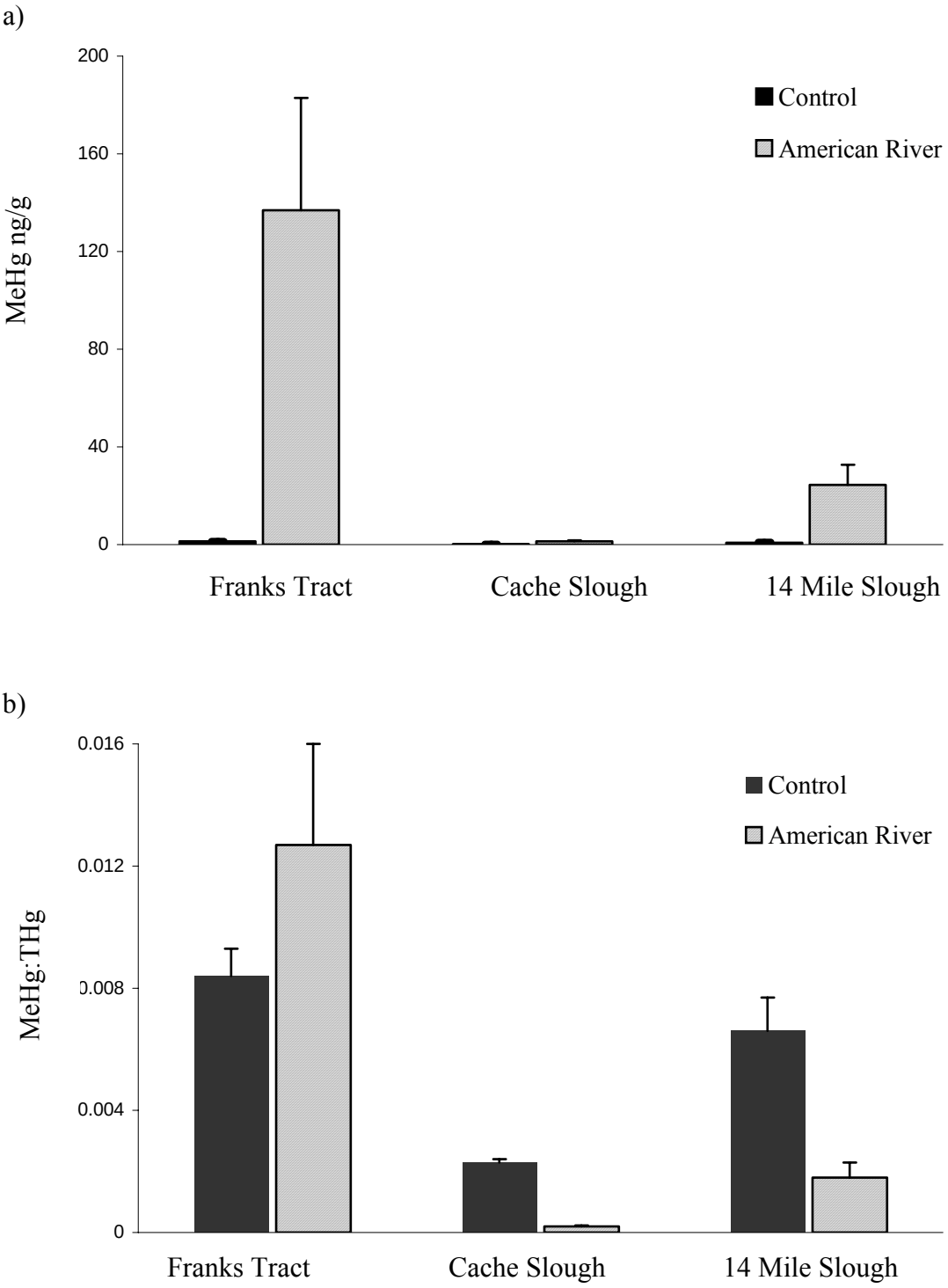
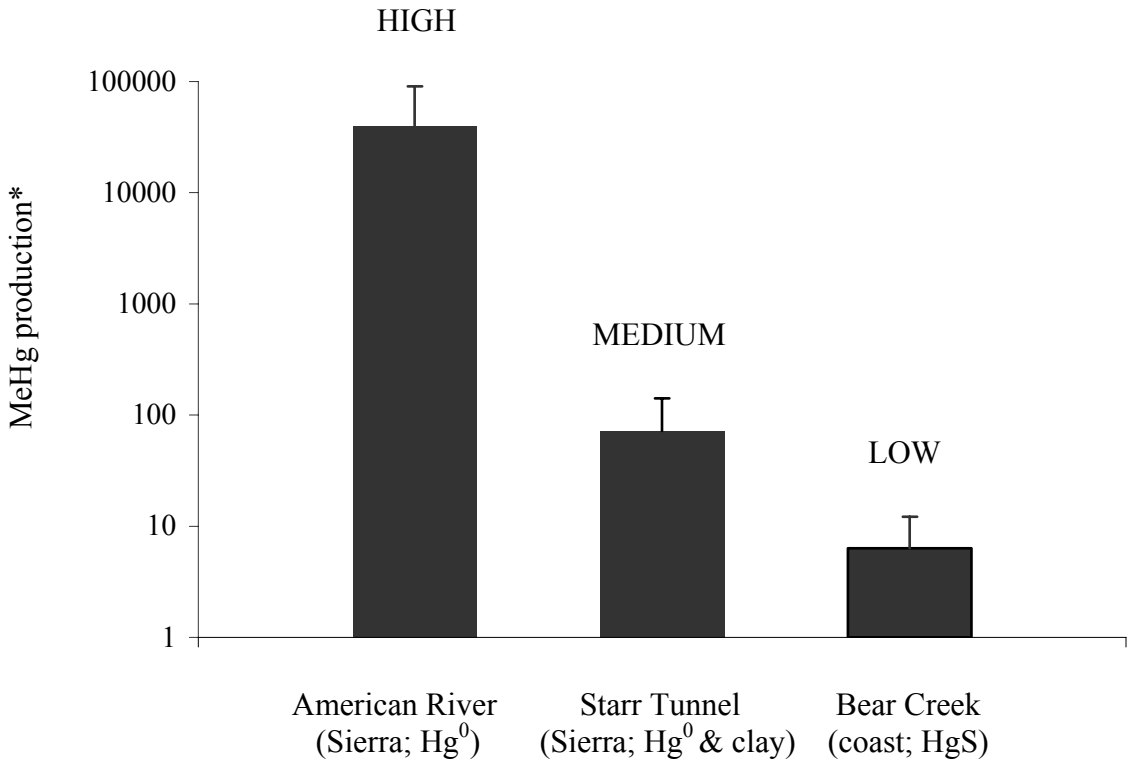


Figure 10. Priority for mitigation of Hg contaminated sediments from mercury and gold mining in California ranked using potential for MeHg production.



* Nanograms of MeHg produced per gram of Hg contaminated sediment (potential MeHg production).

Figure 11. Example profile of THg in sediment used in determining depth of amended Hg and portion to analyze for MeHg (Starr Tunnel amended treatment from14 Mile Slough field methylation experiment Summer 2004).

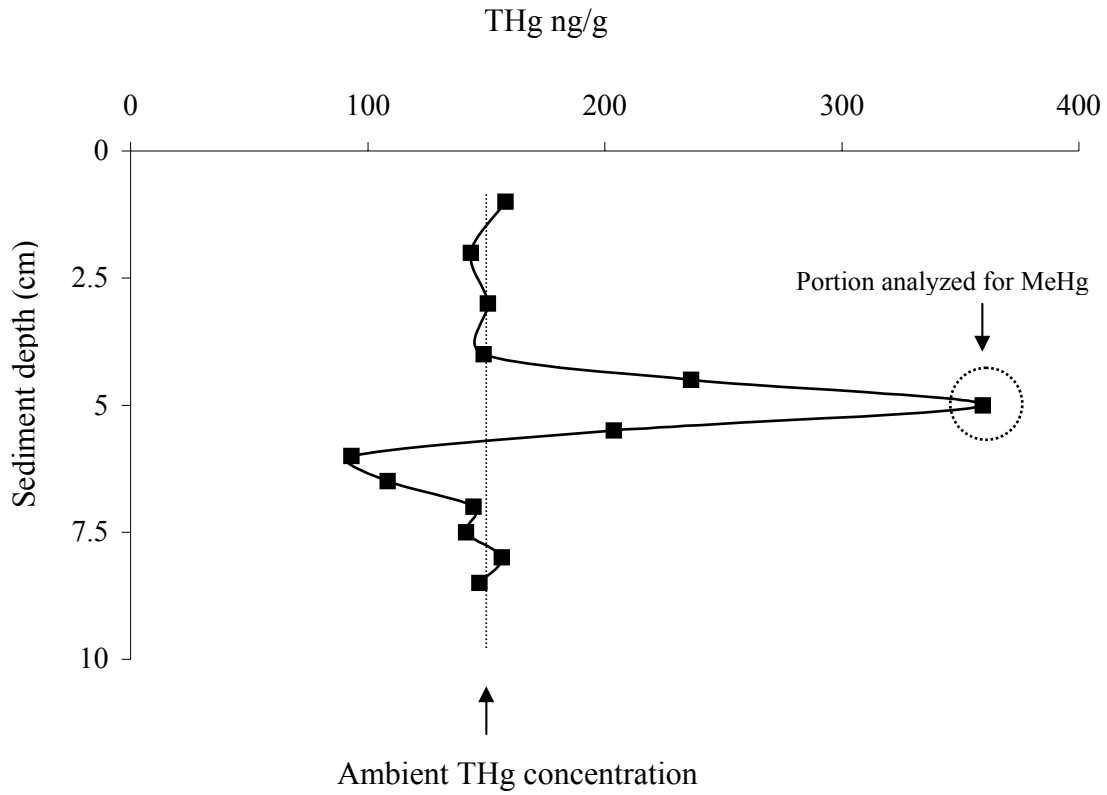


Figure 12. Methylation efficiency in Hg^{2+} amended treatments from laboratory and field experiments for each Delta location arranged by depth (Summer 2004).